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Ueber die Aleukaemische Retikulose* (Letterer-Siwe'sche Krankheit)

Von

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(Received for publication, May 11,1961)

Bei meinen Besuchen im Ausland hat man nicht selten auf meine Autorenschaft der Letterer-Siwe'schen Krankheit Bezug genommen und den Wunsch geaeussert, vom Autor selbst etwas zum Stand der Problematik dieser Krankheit zu hoeren. Ich darf sagen, dass ich selbst erstaunt war ueber die weltweite Diskussion, welche das von mir[1] zuerst als aleukaemische Retikulose beschriebene Krankheitsbild in den Arbeiten der klinischen und pathologisch-anatomischen Forscher gefunden hat. Erst kuerzlich hat Rewald[2] in Mar del Plata (Argentinien) eine ausgedehnte historische Studie und zusammenfassende Darstellung der gesamten Materie geschrieben und bezeichnet, einem Vorschlag englischer Autoren folgend, die Krankheit als Letterer-Christian'sche Erkrankung. Ich muss sagen, dass ich es vom Standpunkt historischer Gerechtigkeit richtiger finden wuerde, von Hand-Letterer-Krankheit zu sprechen, denn Hand[3] war es doch, wenngleich er ihr eigentliches Wesen nicht richtig erkannte, der 1893 eine von ihm fuer Tuberkulose gehaltene xanthoese Retikulose als erster beschrieb, welcher dann spaeter Schueller[4] 1916 die Beobachtung zerstoerender Knochenprozesse hinzufuegte, waehrend Christian[5] 1919 die Trias von Knochenzerstoerung, Exophthalmus und Diabetes insipidus bei dieser Krankheit fand. Daher sprach man dann von Hand-Schueller-Christian'scher Krankheit. Da man heute die von mir beschriebene Retikulose als ein Vorstadium der Hand-Schueller-Christian'schen Krankheit ansieht, so schien der Name Letterer-Christian'sche Krankheit berechtigt. Angelsaechsische, insbesondere amerikanische, Autoren sprechen, der Morphe des mikroskopischen Bildes entgegenkommen, von non lipid und von lipid reticulosis.

Als ich, ein damals noch junger Assistent des Wuerzburger Pathologischen Institutes, im Oktober des Jahres 1923, also vor 38 Jahren, die Obduktion eines sechs Monate alten Kindes vornahm, dessen Leiche mit der Diagnose "Otitis media purulenta, Abszess am Hinterkopf, Sepsis, Purpura, Bronchopneumonie" dem Institute uebergeben worden war, ahnte ich nicht, damit ein Krankheitsbild vor mir zu haben, welches bis zu diesem Zeitpunkt noch nicht beschrieben worden

* Vortrag gehalten am 24. April in der Tohoku Medizinischen Gesellschaft.

war. Aber die histologische Untersuchung liess ein klar charakterisierbares Bild erkennen. Die Vergroesserung der Milz und der Lymphknoten war durch eine teils diffuse, teils herdfoermige Proliferation grosser, protoplasmareicher Zellen hervorgerufen, welche nach Kernbeschaffenheit, Zytoplasma und dessen Beziehungen zu dem retikulaeren Fasernetz als hyperplasierte und proliferierte Retikulumzellen angesprochen werden mussten. Mit der Ausdehnung der Retikulumzellen hielt der Schwund der Pulpazellen der Milz einerseits, der Lymphozyten in den Lymphknoten andererseits Schritt. In der Leber nahmen die Kupfferschen Sternzellen, die wir ja heute zu den Retikulumzellen rechnen, an der Vergroesserung und Vermehrung ebenfalls teil. Sie erschienen nicht selten mehrkernig, entweder der Wand noch anhaftend oder noch frei in der Lichtung liegend. In den Bildern meines Originalfalles erkennt man makroskopisch die Purpura mit dem papuloesen Exanthem, mikroskopisch die monohistiozytaere Proliferation im Papillarkoerper, die Rarefikation der Epidermis mit Hyperkeratose. In der Milz diffuse Retikulozytose und Knoetchenbildungen aus Retikulumzellen im Follikelzentrum. In der Lunge interstitielle Infiltration durch Histiozyten und Rundzellen. Somit handelte es sich um eine systematisierte Retikulose, und wenn man die Retikulumzellen als eine der Quellen betrachtet, von denen sich die Monozyten des Blutes herleiten, so war der Name aleukaemische Retikulose gerechtfertigt. Nach 10 Jahren beschrieb Siwe[6] aus Schweden einen ganz gleichen Fall vom klinischen Gesichtspunkt. Es ist mir nun immer als eine besondere Auffaelligkeit erschienen, dass wir in Deutschland Faelle von Retikulose Letterer so gut wie nicht zu Gesicht bekamen, dagegen sind die Faelle von Hand-Schueller-Christian'scher Krankheit nicht so selten. Ich selbst habe bis vor kurzem nie mehr Gelegenheit gehabt, einen gleichartigen Fall von Retikulose zu obduzieren, und auch die Faelle aus Deutschland, die mir zum Teil zur Begutachtung vorgelegt worden sind, sind ebenfalls gering an Zahl. Erst gegen Ende des Jahres 1960 und zu Anfang 1961 ergab sich der merkwuerdige Zufall, dass bei uns in Tuebingen in kurzem zeitlichen Abstand zwei Faelle von Kindern mit der Diagnose "Letterer-Siwe-Krankheit" zur Obduktion kamen.

In auffallendem Gegensatz hierzu konnten artgleiche Faelle einer aleukaemischen Letterer-Siwe-Retikulose in nicht deutschen Laendern, insbesondere in Amerika, relativ haeufig beobachtet werden. Nach den Angaben der Literatur werden es etwa 60 Faelle sein.

Abt und Denenholtz[7] haben 1936 mit der Beschreibung von 25 Faellen vorgeschlagen, die von mir aleukaemische Retikulose genannte Krankheit mit dem Namen Letterer-Siwe'sche Krankheit zu belegen.

Es wuerde wahrscheinlich ueber die Letterer-Siwe'sche Krankheit gar nichts soviel gesprochen worden sein, wenn nicht ihre Problematik sich in einer ueberraschenden Weise ausgeweitet haette. Diese hat zwei verschiedene Seiten: eine aetiologische und eine morphogenetische. Uber die Aetiologie der Letterer-

Siwe-Krankheit koennen wir heute nur sehr wenig sagen. In meiner ersten Veroeffentlichung habe ich die Vermutung ausgesprochen, dass es sich um die Folge eines chronischen Infektes handeln koennte. Diese Ansicht haben viele spaetere Autoren auf Grund eigener Beobachtung zugestimmt. Oberling und Roussy[8)] haben eine Virusgenese fuer wahrscheinlich gehalten. Ich komme auf die Frage der Aetiologie nochmals zurueck.

Eine grosse Bedeutung kommt auch der morphogenetischen Problematik zu. Denn, wie Sie wissen, betrachtet eine nicht geringe Zahl von Autoren die aleukaemische Retikulose, die Hand'sche Erkrankung und das eosinophile Granulom als verschiedene Erscheinungsbilder ein und derselben Krankheit. Der erste, welcher auf Grund einer eigentuemlichen morphogenetischen Gleichheit der Hauterscheinungen bei Retikulose einerseits und bei Hand'scher Krankheit andererseits diese Ansicht vertrat, war mein jetziger Tuebinger Fakultaetskollege Gottron[9)] im Jahre 1931. Im Laufe der Jahre sind die Stimmen, welche die drei Krankheitsbilder Retikulose, Hand'sche Krankheit und eosinophiles Granulom als eine nosologische Einheit betrachten wollen, immer zahlreicher geworden. Trotzdem kann man auch heute noch drei Meinungen unterscheiden: eine unitarische, eine dualistische und eine trialistische, welche besagen, dass entweder alle drei Krankheiten zur gleichen Einheit gehoeren oder die Retikulose und die Hand'sche Krankheit grundsaetzlich dasselbe bedeuten und schliesslich das eosinophile Granulom auch zu dieser Krankheitseinheit gehoert.

Ich kann mir vorstellen, dass Sie von dem Autor der Letterer-Siwe-Krankheit selbst gerne hoeren wuerden, wie er ueber diese Frage denkt. Ich bekenne, dass ich anfaenglich gegenueber der unitarischen Anschauung im Interesse einer reinlichen Trennung pathologisch-anatomisch erfassbarer Krankheitsmerkmale sehr zurueckhaltend war. Die morphologische Gleicheit ist a priori kein Beweis fuer gleiche Entstehungsgeschichte, denn Isomorphie ist nicht immer gleich Isogenie. Zu dieser Ansicht hat mich auch die Beobachtung gefuehrt, dass das klinische Letterer-Siwe-Syndrom nicht in jedem Fall dem gleichen anatomischen Befund entspricht. Vor einigen Jahren habe ich ein Kind obduziert, welches laengere Zeit mit dem klinischen Letterer-Siwe-Syndrom erkrankt war und dessen Autopsie keine aleukaemische Retikulose, sondern eine aleukaemische Retikulohistioleukoblastose ergab. Dieser Fall hatte histologisch gleiche Hauterscheinungen wie die Retikulose und die Hand'sche Krankheit. Und sogar in der Milz waren retikulozytaere Knoetchen wie bei der Retikulose Letterer und der Granulomatose Hand zu sehen. Niemand wird aber behaupten wollen, dass dieser Fall nun auch zu der grossen Krankheitseinheit gehoert. Es ist vielmehr eine Krankheit fuer sich und zwar eine Blastomatose und zeigt, dass sowohl die einfach proliferierende hyperplastische, wie die blastomatoese Retikulose der Haut zu Purpura, zu Epithelnekrosen und Ulzerationen fuehren kann und dass die Lokalisation dieser Purpura am Gesamtkoerper fuer die Letterer-Siwe-

Krankheit, die Hand'sche Krankheit und die Retikulohistioblastose dieselbe ist. Das heisst also, die klinische Morphologie kann trotz verschiedener Krankheiten die gleiche sein. Auf Grund vergleichender Studien bin aber auch ich heute der Ansicht, dass mindestens die Retikulose und die Hand'sche Krankheit in eine Krankheitseinheit zusammengehoeren. Nach meinem Dafuerhalten ist die Letterer-Siwe-Krankheit als aleukaemische Retikulose "ohne groben Knochenbefall" (Feyrter)[10] als die frueheste und infantile Form dieser Erkrankung aufzufassen, waehrend die Hand'sche Krankheit in einem spaeteren Lebensalter, aber noch immer in der Kindheit, auftritt, und die von Henschen[11] in einer Monographie beschriebenen ossaeren Xanthomatosen Erwachsener das adulte Stadium vorstellen. Dabei ist die Entwicklung von Granulationsgewebe und die verfettende Transformation der Retikulumzellen, welche beide bei der reinen aleukaemischen Retikulose genau so fehlen wie alle Knochenbeteiligungen, nicht allein vom Lebensalter der Kranken und auch nicht allein von der Dauer der Krankheit selbst abhaengig, sondern es scheint, dass auch die Staerke der wirkenden Krankheitsursache eine wichtige Rolle fuer das schliessliche Erscheinungsbild spielt. Je laenger die Krankheit dauert und je juenger der Patient bei ihrem Beginn war, desto mehr entwickelt sie sich von der anfaenglichen hyperplastisch-proliferierenden Retikulozytose ohne allen Knochenbefall, aber mit Hauterscheinungen zur granulierenden und verfettenden osteoklastischen Lipoidgranulomatose und von da zur granulierenden Xanthose des Skelettes ohne alle Hauterscheinungen. Ich muss es unentschieden lassen, ob diese Spaetfaelle auch schon in der Jugend entstanden sind und bis ins Erwachsenenalter latent bleiben, oder ob die Krankheit lebensaltersgebundene Manifestationsformen hat. Beides halte ich fuer moeglich.

Das eosinophile Granulom hat man schon vor den soeben beschriebenen Krankheitssymptomen oder auch erst nach deren Ausbruch beobachtet. Seine Einordnung in zeitlicher und morphischer Hinsicht bleibt nach wie vor schwierig.

Die Lipoidverfettung der proliferierten Zellen und granulierenden Gewebe ist nun a priori kein der Lipoidgranulomatose allein und spezifisch zukommendes Merkmal. Denn es gibt auch Lymphogranulomatosen, die mit der gleichen Lipoidverfettung einhergehen koennen und nur mit einer subtilen Analyse voneinander zu unterscheiden sind. Ferner koennen Retikulumzellknoetchen, wie sie bei der aleukaemischen Retikulose und bei der Hand'schen Krankheit auftreten, auch zu den allerersten und noch nicht charakteristischen Reaktionsformen des lymphoretikulaeren Gewebes ueberhaupt gehoeren und sind somit anfaenglich voellig uncharakteristisch als Wesensmerkmal einer bestimmten Krankheit dieser Gewebe. Als anfaengliche Veraenderungen koennen sie einer Tuberkulose, einem Lymphogranulom, einer Retikulohistioblastose oder einer aleukaemischen Retikulose angehoeren. Ich habe auf diese Verhaeltnisse frueher schon mit einem Hinweis auf unspezifische Fruehstadien der Lympho-

granulomatose aufmerksam gemacht.

Die beiden Faelle, die wir kuerzlich in Tuebingen beobachtet habe, muessen wegen ihres im ersten Fall angedeuteten, aber doch vorhandenen, und im zweiten Fall sehr starken Knochenbefalles schon als Letterer-Christian-Syndrom angesprochen werden und sind daher beide ueber die aleukaemische Retikulose schon hinaus. Indessen zeigen beide eine starke Beteiligung der Haut und des gesamten lymphoretikulaeren Gewebes.

Das erste Kind, ein Maedchen, war bei seinem Tode 9½ Monate, hatte eine sehr starke papuloese Hautretikulose, nur einen kleinen Knochenherd im Schaedel, aber eine auffaellige Retikulose in den Organen, die mit miliargrossen Herden in der Leber deutlich granulierte und sich destruktiv gegenueber dem Lebergewebe verhielt. In der Lunge war nicht wie sonst die Retikulose interstitiell entwickelt, sondern in vielen unregelmaessigen Einzelherden eine retikulozytaere, intraalveolaere, also pneumonische Infiltration entstanden. Das zweite Kind ist nur 2½ Monate alt geworden und hatte schon bei Geburt ein Exanthem. Bei der Obduktion ausgedehnte destruktive Knochenherde von goldgelber Farbe und weicher Konsistenz allenthalben im Skelett, besonders in den Roehrenknochen, einer Beckenschaufel, den Wirbelkoerpern, der Schaedelkalotte und der Schaedelbasis. Splenomegalie mit herdfoermig angeordneten tuberkuliden nekrotisierenden Knoetchen, starke Lymphknotenvergroesserung mit Retikulose und Haemorrhagien. In diesem Fall tritt also die Knochenzerstoerung schon mit Beginn der Krankheit auf, aber das neugebildete Zellgewebe traegt kaum den Charakter des Granulierens und damit des Christian-Syndroms, sondern zeigt eine ueberschiessend proliferierende, den Knochen destruierende Retikulose, wobei trotz des jugendlichen Alter das Skelett schon stark beteiligt ist. Obwohl wegen der vorangeschrittenen Zeit nach dem Tode mit wenig Hoffnung auf Erfolg haben wir versucht, die Retikulumzellen in Milz und Lymphknoten elektronenoptisch darzustellen. Das Ergebnis zeigt eine schon stark vorgeschrittene Autolyse an Kern und Mitochondrien, aber im Zytoplasma eigentuemliche granulierte Koerper von 300 m Durchmesser. Mit allem Vorbehalt stellen wir nur deren Existenz fest, ohne heute schon sagen zu koennen, ob sie native oder Kunstprodukte sind.

Es zeigt sich also, dass fliessende Uedergaenge von retikulozytaerhyperplastischen zu granulierenden Stadien vorhanden sein koennen und die Granulomatose entweder das Merkmal der laengerdauernden Krankheit oder dasjenige seiner Manifestation in erst spaeterem Lebensalter ist. Ich moechte sagen: je staerker die Noxe und je juenger der Patient ist, desto staerker ist die zellige Proliferation; je schwaecher die Noxe und je aelter der Kranke, desto granulierender ist der ganze Prozess. Dadurch aber kommen ganz verschiedenartige Erscheinungsbilder zustande.

Dies zeigt schliesslich noch ein dritter Fall eines Erwachsenen mit Xantho-

granulomatose des Skeletts und des Fettgewebes, den wir kuerzlich obduzierten und der meines Erachtens auch in diese Krankheitsgruppe gehoert. Er war 41 Jahre alt. Seine Krankheit wurde fuer ein Lymphogranulom oder ein Retothelsarkom gehalten. Er hat eine alte, zum Stillstand gekommene Tuberkulose, eine interstitielle Nephritis mit Schrumpfniere und starb an Lungenoedem bei Fettdegeneration des Herzens. Die Obduktion ergab eine generalisierte granulierende Xanthomatose mit verschiedenen Herden im Skelett, im Fettgewebe und im Pankreas. Hier tritt die Retikulose ganz zurueck zugunsten einer granulierenden Xanthomatose, die sich ausser im Knochen auch im Fettgewebequallenthalben ausdehnt. Letzteres kommt auch schon in jungen Jahren vor, und Henschen hat gleichartige Fettgewebsherde einmal bei einem nur drei Jahre alten Kind mit Christian-Syndrom gesehen.

Ich moechte nun abschliessend noch einiges zur Aetiologie sagen. Die morphogenetische Symptomatik, die in einer genuegend grossen Zahl von Faellen gut analysiert ist, wird uns im Hinblick auf die Aetiologie nicht mehr viel weiterfuehren. Eine mikrobische Ursache scheint mir trotz des Vorhandenseins gelegentlicher Staphylokokkeninfekte kaum die ausloesende Ursache, sondern nur Begleiterscheinung zu sein. Aus Vergleichen mit anderen Erkrankungsbildern moechte ich vielmehr der Meinung von Oberling und Roussy beipflichten, die fuer die aleukaemische Retikulose eine Virusaetiologie annehmen. Wir haben mit Geweben aus der Milz auch geprueft, ob sich an Gewebekulturen ein zytopathogener Effekt erzielen laesst (Prof. Schaefer, Max-Planck-Institut fuer Virologie, Tuebingen), jedoch mit negativem Erfolg. Auch hier war die uns gegebene Entnahmezeit des Materials nach dem Tode zu lang. Wenn es sich aetiologisch um ein Virus handelt, so ist es m.E. moeglich, dass ein Infekt lange Zeit latent bleiben kann und dass andererseits das Lebensalter fuer den Infektverlauf eine Rolle spielt. Ich habe bestimmte Vorstellungen darueber, auf die ich jetzt in extenso hier nicht eingehen kann. Da wir aber heute ganz andere Moeglichkeiten haben, die Virusaetiologie einer Krankheit zu studieren, so waere es mein Wunsch, dass alle Forscher dieselben im Hinblick auf die aleukaemische Retikulose ausnuetzen wuerden, indem man mit bioptisch gewonnenem Material aus Haut und Knochenmark die noch ungeloeste Frage der Aetiologie der Retikulose und der Granulomatose vom Typ Letterer-Christian sowohl elektronenoptisch wie virologisch zu klaeren versucht. Wollen wir hoffen, dass man bald zu einem Erfolg kommt.

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Changes in Constituents in Arterial Blood Following Experimentally Induced Epileptic Convulsion in Dogs

By

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(Received for publication, May 16, 1961)

Many clinical and experimental studies on the blood following the convulsion in man[2,6,7,27,38] and animals[5,11,25,29,37] are already reported. It seems important to investigate the changes in the milieu intérieur, as they have influences upon the function of various organs, especially of the brain. However, only few attempts have been made to study the progressive changes of the metabolism following convulsions.[7,27,37]

In view of the present situation of the studies on the epileptic seizures, we attempt to investigate the changes in some constituents in the blood successively drawn from the artery. This enables us to have further knowledges on the metabolic changes and the regulation which may play a significant rôle in the recovery course following the nervous and humoral activation caused by electrical stimulation of the brain.

METHODS

Healthy dogs, varying in weight from 10 to 15 kg, were used for these experiments. The food was withheld for 24 hours before the experiment. The animal was placed in supine position and the blood samples were collected from femoral arteries under local anesthesia. For the electrical stimulation two round silver electrodes of 1.5 cm in diameter were applied to the skin about 1.5 cm superior to the eyes. The hair in these regions was previously cut to obtain the contact. The current used was 50 c/s AC of 50 and 100 V for five seconds. By such procedure the typical grand mal reactions as described by Feldman and other investigators[11,20] could be obtained.

From the femoral arteries 20 to 25 ml of blood for various determinations was drawn at certain intervals into syringes whose inner surfaces were previously

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coated with paraffin oil. The blood was soon poured into flasks which contained sodium oxalate (1 mg per ml of blood) and paraffin oil, and were cooled under 7°C. A portion of cooled blood was immediately centrifuged without contact with air for the estimation of pH, phosphate and chloride in the plasma. Double oxalated blood was used for the measurement of the hematocrit value.

For control experiments in which no electrical stimulation was applied, the determination of the same constituents was carried out in five dogs under same experimental conditions to study the effect of successive blood sampling without convulsions.

The following constituents of blood samples were determined.

1. The relative volume of cells and plasma. This was determined by centrifugation of double oxalated blood for 30 minutes at 3,000 r.p.m. using a hematocrit of Hedin type.

2. pH. For the estimation of the hydrogen ion concentration in the plasma, quinhydrone method was used.

3. Chloride. The chloride concentration in the whole blood and the plasma was determined by Schales and Schales method.[35] Chloride in cells was calculated as same as that of phosphorus mentioning below.

4. Phosphorus. Inorganic and acid-soluble organic phosphorus in whole blood and plasma were measured colorimetrically by the method of Fiske and Subbarrow.[13] Attentions were payed to prevent the decomposition of acid-soluble organic phosphorus. Thus, the concentrations of phosphorus in red cells were calculated according to the following formula:

$$Cc = \left(Cb - Cp\,\frac{100-H.C.}{100}\right)\frac{100}{H.C.}$$

where

H.C. = hematocrit value

Cc = concentration in red cells(mg/dl)

Cb = concentration in whole blood(mg/dl)

Cp = concentration in plasma(mg/dl)

5. Blood sugar. The sugar concentration in the arterial blood was determined by Hargedorn-Jensen's method.

RESULTS

1. Hematocrit value. In Table I, hematocrit value of convulsed and non-convulsed dogs are shown. In non-convulsed dogs variations in the hematocrit readings for about two hours remained 1. On the other hand, in convulsed dogs, a significant increase in the readings of cell volume (3–10, mean value 5) was observed at 3 to 10 minutes and returned to normal at about 35 minutes after the convulsion. This increase in the cell volume was also found in anesthetized, splanchnicotomized or adrenalectomized dogs following the convulsion. In a

TABLE I. Hematocrit Values in Control and Convulsed Dogs.

	Sample	1	2	3		4	5	6	Remarks
	Time (min)	30-60	5-150	3		10	35	65	
Animal number	No.9	53	53	52		52	53	53	Control
	No.10	47	46	47		47	47	—	Control
	No. 3	37	38	42	Convulsion or injection	39	37	—	100V
	No. 4	38	39	41		41	38	—	100V
	No.24	39	38	43		42	40	36	100V
	No. 7	44	43	46		46	44	43	50V
	N0.26	32	31	36		36	31	31	50V
	No. 5	46	46	55		55	53	50	Adrenaline injection
	No.18	37	—	47		—	—	—	splanchnicotomized (100V)
	No.21	38	37	42		—	36	—	Adrenalectomized (100V)
	No.23	38	43	48		—	44	44	Splanchnicotomized-& adrenalectomized

No. 21 and No. 23 were stimulated at 100 V under barbiturate narcotization.

dog into which 10 gamma/kg of adrenaline (Park Davis) was intravenously administered, the cell volume rose also remarkably, having sustained for 10 minutes, then fell gradually, and remained at a higher value than that in control period even at 60 minutes after the convulsion.

2. pH. The representatives among 14 convulsed dogs are demonstrated in Table II. A slight decrease in the plasma pH was found during 90 minutes by sampling of several specimens in control dogs. In convulsed dogs, however, pH of the plasma reached abruptly to the minimum value between 2 and 4 minutes and returned to normal at about 30 to 60 minutes after the convulsion. These decreases in the plasma pH were ranged between 0.12 to 0.18, and were significant since variations in pH during control period were ranged between 0.03 to 0.07. A marked fall of the plasma pH (from 7.55 to 7.34) was induced by the intravenous administration of adrenaline, but disappeared within 10 minutes following the injection.

3. Inorganic and acid-soluble organic phosphorus. In preconvulsive period the concentration of inorganic phosphorus varied between 2.1 and 3.8 mg/dl in the whole blood, 2.3 and 4.5 mg/dl in the plasma, 1.3 and 3.2 mg/dl in red cells,

TABLE II. pH Values in Control and Convulsed Dogs

	Sample	1	2		3	4	5	6	Remarks
	Time (min.)	30-35	5-10	0	3	10	30-35	60-65	
Animal number	No.9	7.41	7.41	None	7.39	7.39	7.39	7.34	Control
	No.10	7.40	7.40		7.41	7.37	7.37	7.38	Control
	No.7	7.68	7.64	Convulsion	7.50	—	7.68	7.68	Convulsed
	No.8	7.49	7.49		7.37	7.39	7.44	7.48	Convulsed

the mean values being 3.1, 3.6 and 2.5 mg/dl respectively. The concentration of acid-soluble phosphorus was negligible in plasma, and 11.1–28.6 mg/dl in whole blood, 23.1–50.0 mg/dl in cells, their mean values in six dogs being 16.2 mg/dl and 34.2 mg/dl respectively. These values were similar to those reported by others.[31,32] In control dogs, the variations of the concentration of inorganic and acid-soluble phosphorus in the blood, plasma and cells remained within 20% of the initial values during successive determinations for 90 minutes (Fig. 1).

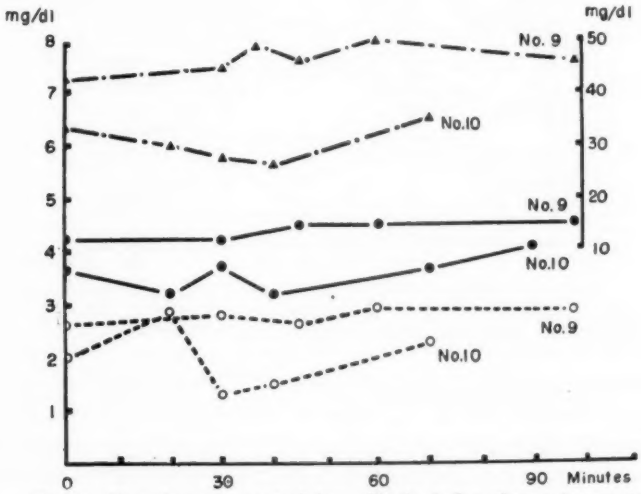

Fig. 1. Phosphorus in control dogs. Black circle; plasma inorganic phosphate. Hollow circle; cell inorganic phosphate. Triangle; cell acid-soluble organic phosphorus (scale on the right).

As shown in Fig. 2, a remarkable increase in the concentration of blood inorganic phosphorus (33.3–93.4% in whole blood, 33.3–70.0% in the plasma, 30.6–49.2% in the cells) was observed after the convulsion. Only in a case a decrease (38.0% decrease of the initial value), which was found after an initial rise, was obtained at 60 minutes following convulsions. In most cases, the maximum values were found at about 30 minutes after the convulsion, and returned to the initial value at about 2 hours following the electrical stimulation.

On the other hand, the changes in the concentration of acid-soluble phosphorus in cells remained within the range of variations during control period.

The changes of phosphorus compounds in the blood caused by the injection of adrenaline were rather complicated. A significant decrease in inorganic phosphate was found immediately after the administration. This decrease was more striking in the plasma (28.2%) than in the cells (18.6%), but the relation

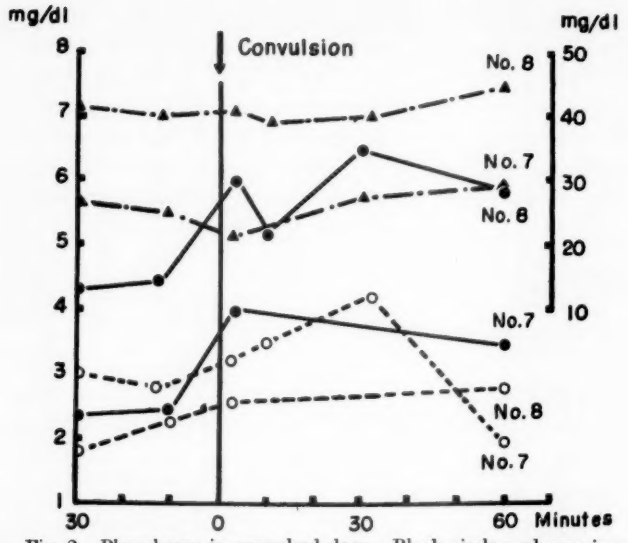

Fig. 2. Phosphorus in convulsed dogs. Black circle ; plasma inorganic phosphate. Hollow circle ; cell inorganic phosphate. Triangle ; cell acid-soluble organic phosphorus (scale on the right).

in the decrease was reversed after 10 minutes following the convulsion, i.e. 48.6% decrease in the cells and 24.7% decrease in the plasma. Acid-soluble organic phosphorus in whole blood increased up to 30.9% within 10 minutes after the injection, though no significant change of its concentration in the cells was found.

4. Chloride ions. The concentration of chloride ions in blood of four control dogs were agreed with that reported by other authors,[8] being 84.1–96.2 mequiv/l (mean, 89.6 mequiv/l) in blood, 109.3–120.6 mequiv (mean, 113.6 mequiv/l) in the plasma, 54.6–67.6 mequiv/l (mean, 57.4 mequiv/l) in the cell. The variations in both the plasma and the cell were within about 5% of the initial values during the control experiment for 90 minutes (Fig. 3).

Chloride in blood showed a significant increase following the convulsion. It showed the maximum increase at 2–3 minutes after the convulsion and went back to normal within 60 minutes after the stimulation. The rate of change of the chloride concentration in the cell was larger than that in the plasma, therefore the Donnan's ratio of chloride showed a slight increase following the convulsions. The recovery period of the concentration of chloride in the cell to the preshock value was longer than that in the plasma.

5. Blood sugar. The sugar in control blood varied from 83 to 140 mg/dl with a mean of 111 mg/dl in six normal and two narcotized dogs. The fluctuation of the blood sugar level remained within 10 % of the initial value in

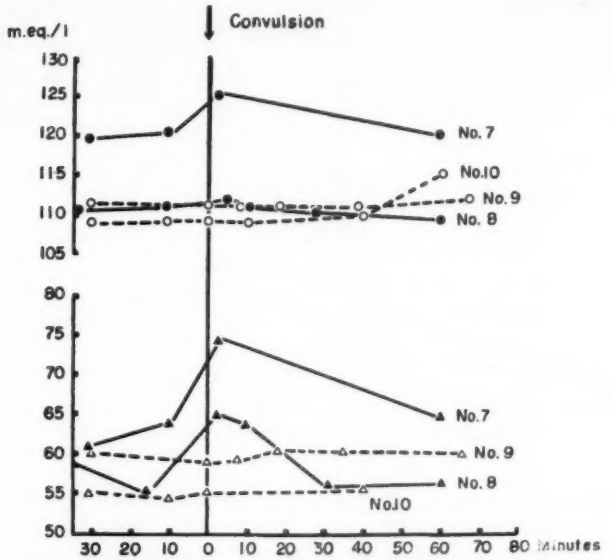

Fig. 3. Changes in concentration of chloride in plasma (above) and cells (below) induced by convulsions. Black and hollow circles ; plasma chloride in convulsed and control dogs respectively. Black and hollow triangles ; cell chloride in convulsed and control dogs respectively.

each dog, whether the animal was normal or narcotized, except one dog in which it attained as much as 15.6% of the initial value.

The results obtained from the non-narcotized convulsed and control dogs are illustrated in Fig. 4. The rise in the blood sugar reached to the maximum value (from 35 to 50% increase) within 2 minutes after the convulsion and then slowly declined. In a case, this rise was sustained at almost the same level over a period for 60 minutes. As seen in Fig. 5, the elevation of the sugar level in narcotized convulsed dogs was generally lower than that in non-narcotized dogs, although they were stimulated by the electric current of the same intensity. This difference can be explained partly by the fact that the grand mal reactions in narcotized dogs were less than those in non-narcotized dogs (see DISCUSSION). The maximum per cent increase in narcotized dogs was ranged between 29.0 and 33.3%.

The blood sugar in splanchnicotomized animals was elevated as high as that in normal animals following convulsions, but in a dog denervated more than a week before the experiment, it appeared to return to its initial value earlier than that in the intact animals. On the other hand, only a slight and transitory increase, which was within the range of variations in control value, was found

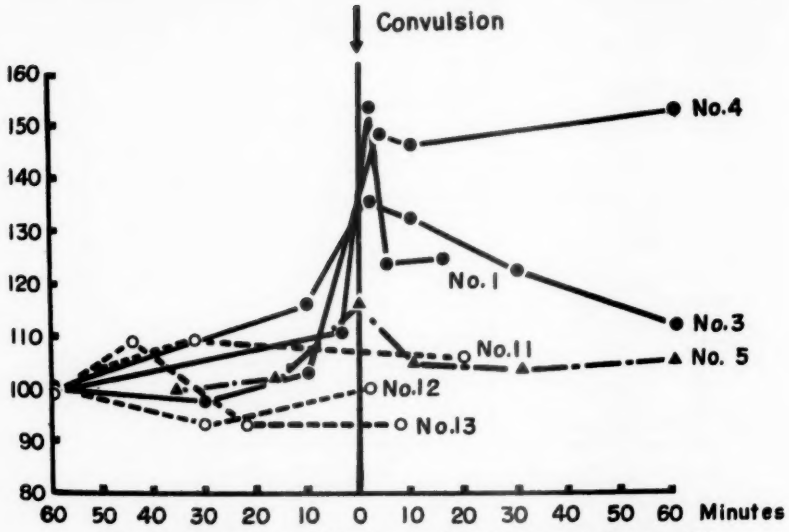

Fig. 4. Comparison of blood sugar level in convulsed dogs with that in non-convulsed dogs (expressed as per cent of the initial values). Black and hollow circles ; convulsed and control dogs respectively. Triangle ; non-convulsed dog which was adrenalectomized and splanchnicotomized 3 hours before the first determination of the blood sugar.

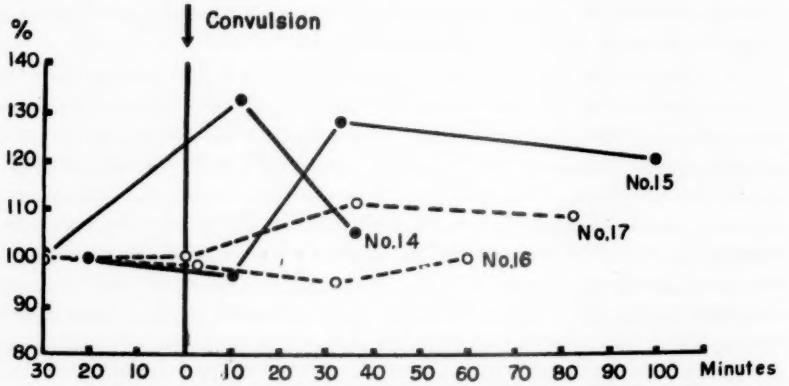

Fig. 5. Changes in blood sugar in narcotized dogs. Black circle : convulsed dogs. Hollow circle ; non-convulsed dogs.

in four adrenalectomized dogs, whether they were splanchnicotomized or not. Moreover, in an adrenalectomized dog, about 25% decrease was induced by the convulsions. These findings show that the adrenals play a significant rôle

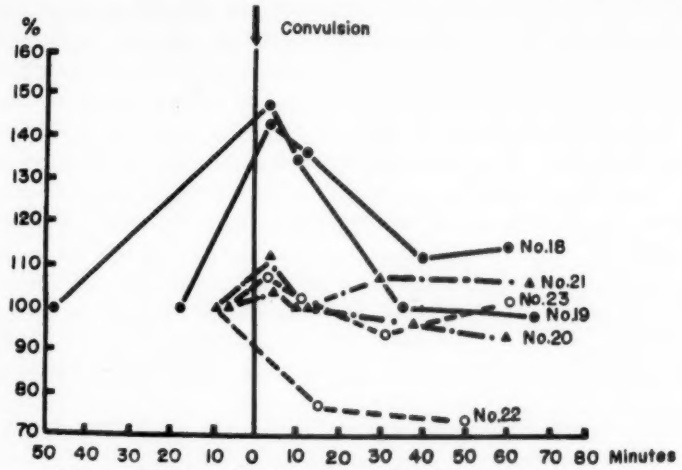

Fig. 6. Effect of splanchnicotomy and adrenalectomy on changes of blood sugar caused by convulsions. Black circle ; splanchnicotomized dogs. Hollow circle ; adrenalectomized dogs. Triangle ; adrenalectomized and splanchnicotomized dog. Operations of animals were finished 3 hours before the electrical stimulation except No. 19 which was splanchnicotomized 10 days before the experiment. No. 21 and 23 were stimulated under narcotization.

for the remarkable elevation of the blood sugar level following convulsions, and that the impulse through the splanchnic nerve may have only some summative or prolonging effect on the rise in the blood sugar by convulsions.

DISCUSSION

It has been indicated that the autonomic response to the electroshock is a non-specific reaction originated from diencephalo-hypophysis and hyperactivity of neuro-vegetative system, and is characterized by a 'syndrome sympathique médullosurrénal' as in the case of defense reaction or emotional excitement.[7,11,25,29] On the other hand, the convulsion induces an anoxic state due to violent muscle exertion with transient apnea as in the case of hard exercise or severe hemorrhagic shock. Therefore, the general metabolic changes in convulsions can be compared with those obtained from the experiments on the emotional excitement, hard exercise and also traumatic hemorrhagic shock.

It has been reported that the rise in blood sugar by stimulation of hypothalamic nuclei[28] and piqûre diabétique[22] and by reflex[18] was much reduced by denervation of the adrenals. The remained slight rise in the blood sugar is thought to be mainly due to the liver activated by impulses through the hepatic nerve. Same conclusion was presented from the experiment on hyperglycemia

induced by emotional excitement.[4] However, it will be recalled that the splanchnicotomy is not a sufficient denervation of the adrenals in dogs.[39] Accoding to our results in convulsed dogs, little change in the rate of increase in the blood sugar level was found after the section of the splanchnic nerves as is reported in the case of asphyxia,[24] while no significant rise was obtained in adrenalectomized dogs. These findings seem to support the view that the marked rise in blood sugar following convulsions may be mainly due to the direct stimulation of adrenals by anoxemia suggested by Malméjac, Chardon and Gross (1950).[30]

As mentioned above, the degree of the rise in blood sugar following convulsions was less in narcotized dogs than in non-narcotized ones. This might be also due in part to the possibility that the anoxemia by weaker grand mal reaction in the narcotized dogs is caused to a lesser extent than in non-narcotized ones.

The rise in the blood sugar by convulsions was much more intense and prolonged than that induced by intravenous injection of adrenaline of 10 gamma/kg. It may be possible that 10 gamma/kg adrenaline is overdose for the induction of the maximum hyperglycemia in dogs, since it has been reported that the most effective dose is 4 gamma/kg/min in cats.[18]
Moreover, it must be kept in mind that the abrupt fall in the blood sugar following convulsions in adrenalectomized dogs can be due to waste of sugar by muscle exertion and also partially by activation of vago-insulin system.[10,11,25]

The increase in the hematocrit readings and the decrease in the glomerular filtration rate[26] following convulsions, which are explained to be related to the activation of splenic and renal nerves by the stimulation of sympathetic system,[3] disappeared within 20–30 minutes after the stimulation. In the case of emotions[34] and also of convulsions,[26] an increase in the reabsorption of water in renal tubules due to the increased secretion of ADH was suggested, and it seems to be possible that the liberation of anterior pituitary hormones, especially ACTH, is accerelated directly (via diencephalon) and indirectly (by the increased secretion of adrenaline) and then the secretion of glycocorticoids is induced. Those may co-operate for the prolonged elevation of blood sugar level with the action of the hormone from the adrenal medulla.

Some authors reported that the decrease in plasma inorganic phosphate following the initial rise was observed within 45 minutes after hard exercise for short period in man and animals.[10,15,17] According to their results, it takes more than two hours for the recovery of this decrease in the concentration of inorganic phosphate. In our results, only in a few cases of narcotized and weakly convulsed dogs, such a fall in plasma inorganic phosphate was noted at 10–20 minutes after the convulsions. And the extent of the rise in blood sugar was slight. However, the concentration of inorganic phosphate in the plasma following

typical convulsions increased and sustained for more than an hour in most cases. The same pattern of changes in inorganic phosphate was reported by Ward and Call[37] in convulsed rats. This evidence shows that the typical convulsions are characterized by an abnormal metabolic state accompanied by an extraordinary oxygen debt as the severe hemorrhagic shock,[1,33] and is explained to be due to the inhibition of phosphorylation. Moreover, this seems to support the view that the convulsions and the severe hemorrhagic shock have many common features in the metabolic state of organs.

The metabolic acidosis is caused by experimentally induced convulsions in rats.[37] In our experiments the same state was found in convulsed dogs by determining pH and also carbon dioxide content in blood. Changes in the acid-base balance is one of the factors which may have influences upon the functions of organs, especially of the central nervous system, since the excitability of neurones was changed by a shift in pH[9] and be counted up one of the reasons for the threapeutic effect of convulsions.

Plasma pH and plasma carbon dioxide content, though the latter was determined in only four cases, changed in parallel. They showed the minimum values at 2–3 minutes after the convulsion and gradually returned to normal from 30 to 60 minutes after the electric shock. Although the hyperpnea which helps the driving carbon dioxide out of the blood begins to appear immediately after the termination of the convulsions, it is not sufficient to avoid the metabolic acidosis caused by an anoxia. The concentration of inorganic phosphate in the plasma did not change in the same way as pH in the plasma. This seems to indicate that the concentration of lactic acid in blood may mainly be related to couple with the acid-base balance.

As described above, such an increase in the concentration of chloride in blood of dogs was found following convulsions, as reported in the case of hard exercise in man and animals.[10,15] It is worth mentioning that the rate of increase in chloride in the cell was much larger than that in the plasma, in other words, there was an increase in Donnan's ratio which indicates chloride in the plasma moves into the cell as if there is some mechanism to maintain the concentration of plasma chloride constant. One of the factors concerning this mechanism may be metabolic acidosis mentioned before, since the increase in Donnan's ratio run parallel with the decrease in plasma pH. In connection with this, it will be remembered that the same pattern of chloride shift is aroused during the course of general adaptation syndrome.[23] With reference to the view described by Fenn,[12] such an increase in plasma chloride is accounted for the shift of water from the extracellular space to the intracellular space as the result of the rise in the intracellular osmotic pressure of muscles by contraction of muscles during grand mal reactions.

Rapoport and Guest[31] reported that the concentration of cell acid-soluble

organic phosphorus depends upon the factors which have influences on the enzyme system of glycolysis in red cells, that is, blood pH, the concentration of plasma inorganic phosphate and of chloride in the cell. Thus the concentration of cell acid-soluble organic phosphorus increases with the rise in the concentration of plasma inorganic phosphorus, while the former decreases with the diminution of blood pH and with the increase of cell chloride concentration. In our convulsed dogs, little change in the concentration of cell acid-soluble organic phosphorus was found. This can be explained by supposing as the result of the balanced state of these three factors.

SUMMARY

1. Hematocrit value, pH, chloride, sugar, inorganic phosphate and acid-soluble organic phosphorus in arterial blood of dogs were successively determined before and after the electrically induced convulsions and the following results were obtained.

2. The hematocrit value increased abruptly within 2–3 minutes and sustained the maximum value for more than 10 minutes, then returned towards normal at 30 minutes after the convulsion.

3. pH in the plasma decreased, attaining the minimum value at about 2–3 minutes and returned to normal by 60 minutes after the convulsion.

4. The concentration of chloride in the blood increased soon after the electroshock, and showing an increase in the Donnan's ratio, then returned to the initial value within 30–60 minutes following the electrical stimulation.

5. A marked rise in the blood sugar was found immediately after the convulsion and then slowly decreased, but it remained at a level higher than the initial one even 60 minutes after convulsions. This rise was little affected by the bilateral splanchnicotomy, but almost abolished after adrenalectomy.

6. The concentration of the plasma inorganic phosphate increased remarkably after the convulsion, showing the maximum value at about 30 minutes, then gradually diminished, but did not return to normal even at 60 minutes after the convulsion. On the other hand, cell inorganic phosphate showed a slight increase. No significant change in the concentration of acid-soluble organic phosphorus in cells was found.

7. The changes in the constituents of arterial blood by electric convulsions were discussed with regard to the results obtained from the experiments on the emotional excitement, hard exercise and hemorrhagic shock in man and animals.

The outline of this work was reported in the 28th General Meeting of Japanese Physiological Association (1951).

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Prolactin-Like Substance of Human Placenta

By

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INTRODUCTION

Lactation is one of the most complicated physiological process carried out in living female mammals, and some hormones and several neural factors are mobilized in the process. The correlation between the placenta and lactation has been noted for a long time, as suggested by the avidity of mother mammals in eating up the expelled placenta. Halban[1] (1905), basing himself mainly on clinical observations, reported the existence of a substance in the ovary that stimulates the growth of the mammary glands but is not capable of causing lactation by itself, and predicted the discovery of some specific substance which stimulates the change of mammary gland by pregnancy in the syncytial cells of the placenta, thus foretelling the establishment of the endocrine nature of the placenta. Lactation, he says, begins with the expulsion of the placenta, that is, with the cessation of the endocrine secretion by it.

Since then, many works have been done on the mechanism of lactation and the lactogenic substances, but too many points of these problems are regrettably still in a shorud of darkness. The existence of some substance inciting lactation in the anterior pituitary lobe was proved by Stricker and Grüter[2] (1928), who succeeded in inducing lactation in pseudo-pregnant rabbits by injecting extract of the pituitary gland, and has been confirmed by many experimental studies since then. Riddle *et al.*[3] (1932) discovered that this lactogenic substance stimulates the crop gland of pigeon and entails crop lactation, and christend the substance "Prolactin". Ehrhardt[4] (1936) reported that this prolactin may be demonstrated in the human placenta in early pregnancy but not in mature placenta, while Ito[5] (1953) extracted the crop-reaction positive substance from human placenta and asserted that it is contained particularly rich in the mature placenta. Of course, there is a large camp of researchers who deny the existence of any lactogenic substance in the placenta. The present author also undertook

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a study on this problem of lactogenic substance, using human placentae of various stages of pregnancy for experimental subcutaneous grafting near the crop of pigeons, and for testing the crop reaction following administration of their extraction in saline; similar tests were made with extracts prepared following some methods formulated by past authors for making pituitary extracts as well as a method of my own. Finally the chemical characteristics of the substance were also determined.

METHODS

1. Crop reaction with crude extracts

Samples of human placentae of various stages of pregnancy were treated as described in "Results", and the crop reaction was tested. Extracts were injected near the crop either intra- or hypodermally, or less frequently in the pectoral muscle once daily for 4 days with autopsy 24 hours after the last injection. In case of necessity, a portion of the crop-glands was cut out, fixed in 10 % formalin solution and histologically examined after hematoxylin-eosin staining. The methods of experiments with rabbits and human subjects are given in the appropriate paragraphs in "Results".

2. Our new method for extraction

Next, the author made the experiments described below, utilizing the method applied by Yoshizaki[9] in his experiments for extracting the factor causing toxemia of pregnancy, which is useful in easily obtaining relatively large quantities of extracts. Fresh full-term placentae were washed, shredded, freed of blood with a press as far as possible, minced and mixed and stirred in physiological saline for extraction at low temperature. The precipitate (Fraction I) produced when the pH value of the extract was lowered to 4.2 was stirred in 3 mol saline at pH 7.0, the pH value was again lowered to 4.2, and from the precipitate produced, Protein I was isolated. The supernatant remaining after separating Fraction I above was adjusted to pH 7.0, totally saturated with ammonium sulfate, the precipitate produced hereby was throughly dialysed in running water and the precipitate produced at pH 5.8 (Fraction II) was processed, Protein II being obtained. The supernatant after elimination of Fraction II was repeatedly mixed with HCl to lower the pH value to 2.0 and with $Ba(OH)_2$ to raise it to 9.0, for eliminating nucleic acid, freed of Ba with H_2SO_4 mixed with alcohol to 15%, and Protein III was obtained from the precipitate produced (Fraction III). The recovery rate was 40 mg. each of Proteins I and II and 20 mg. of Protein III.

RESULTS

1. Crop reaction with crude extracts
   1) Crop reaction after subcutaneous transplantation of human placental grafts

Fresh decidua, chorion or entire placenta was thoroughly rinsed in water, to remove all blood, repeatedly pressed between filter papers to draw off water, weighed and grafted beneath the skin near the crop of pigeons, and the reaction was evaluated on the 5th day. For control tests, uterine muscles or subcutaneous fat was similarly grafted. Implanting of a placental sample of any stage of pregnancy caused crop reaction similar to that at the time of incubation and nursing youngs, consisting in hyperemia, thickening and opacity of the mucous membrane. In the control tests of grafting uterine muscles and subcutaneous fat too, capsules were formed around the graft by aggregated connective tissue and the crop's mucous membrane was thickened and turned opaque.

2) Crop reaction after injection of placental extract in saline

Fresh samples were mixed with double volume of physiological saline, left standing for a night, centrifuged, and the supernatant was kept in ice-box. After daily injection of this extract subcutaneously near the crop for 4 days, the reaction was evaluated on the 5th day. The extract from placenta of the early stage caused slight vascular dilatation in the mucous membrane, but that from full-term placenta caused no reaction.

3) Crop reaction after administration of other extracts

For preparing the extract, placentae sampled at full-term delivery were subjected to the process suggested by Bonsnes[6], Li[7] and Lyons[8]. With some fractions, mild congestion and thickening were caused in the mucous membrane, but in any case, the reaction was too weak to be called positive.

2. Experiments with extracts by our new method

1) Crop reaction in pigeons

a) After intradermal injection

When any of the fractions above was injected into the skin over the crop, the result was always positive. The per pigeon unit equivalent was 2 mg. with Protein I, 1 mg. with Protein II and 1/2 mg. with Protein III, that is, the pigeon unit per 1 mg. of fraction was 1/2 with Protein I, 1 with Protein II and 2 with Protein III (Table I).

Table I. Crop-Reaction after Intradermal Injection

No. of pigeon	Body weight (g.)	Extract	Dose (mg.)	Response	Pigeon unit (mg.)
1	290	Protein I	1	−	1/2
2	340		2	+	
3	310		4	+	
4	320	Protein II	1/2	±	1
5	340		1	+	
6	350		2	+	
7	310	Protein III	1/4	±	2
8	285		1/2	+	
9	300		1	+	

b) After intramuscular injection

As by the intradermal injection method the possibility of non-specific inflammatory reaction due to impurities is not excluded, the crop reaction was tested also by intramuscular injection of the same fractions in the pectoral muscles. Positive results were obtained after injection of 20 mg. of Protein I and 10 mg. of Protein II or III. The epithelium of the crop mucosa formed large folds, many downgrowths of germinative zone were found and the connective tissue layer forming the tunica propria was found thinned down. Some of the enlarged folds tended to form a plateau by mutual fusion (Figs. 1, 2, 3 and 4).

2) Experiments with rabbits

a) By Meites and Turner's[10] method

Rabbits on the 15th day of pseudopregnancy were injected with the tested protein fractions in the lactiferous tubuli under ether anesthesia, and sacrificed 5 days thereafter for testing the milk production. As shown in TABLE II, milk could be actually pressed out in one case only after 5 mg. of Protein II. After Protein II, the lactiferous tubules were strongly proliferated, the terminal apparatus were aggregated into glandular lobules and fat drops were found in the lumina of the terminal alveoli (Figs. 5, 6 and 7).

TABLE II. Meites and Turner's Method

No. of rabbit	Body weight (g.)	Extract (mg.)	Milk secretion	Histological finding
1	2500	Protein I 2.5	–	Lobules (+) Terminal alveoli (–)
2	2500	Protein I 5.0	–	Fat drops (–)
3	3100	Protein II 2.5	–	ditto
4	2500	Protein II 5.0	+	Terminal alveoli (+) Fat drops (+)
5	2700	Protein III 2.5	–	Lobules (+) Terminal alveoli (–)
6	2200	Protein III 5.0	–	Fat drop (–)
7	2900	Saline 1cc	–	ditto

b) Experiments with rabbits after throwing

Adult rabbits of 2–3 kg. body weight and having experiences of throwing but with their lactation completely suspended after lapse of more than one month after the last milking were injected with the above protein fractions once a day for a week, and the effect on their mammary glands and milk production was studied. As shown in TABLE III, all the fractions were found to cause lactation, for after Protein I milk could be pressed out from the 5th day, after Protein II from the third day and after Protein III from the second day onwards, and even after completion of a series of injection for a week, milking remained possible for 4 days, 6 days and 4 days with each case, respectively. In these cases, histological

TABLE III. Experiments with

No. of rabbit	Body weight (kg.)	Extract (mg.)
1	2.8	Protein I 35
2	3.1	Protein II 35
3	3.0	Portein III 35
4	2.9	Saline 7cc

examination revealed high proliferation of the terminal alveoli and presence of numerous fat drops (Figs. 8, 9 and 10).

3) Effect on lactation in human subjects

a) Method of measuring lactation

The total weight of the suckling in draper just before and after sucking was weighted under precisely the same condition and the difference in weight was taken to represent the quantity of milk ingested in one sucking. At every lactation, the process was repeated and the total daily quantity was computed.

b) Application of fractions

Puerperae free from notable complicating diseases were indiscriminately injected with 200 pigeon units each of the fractions for 5 consecutive days, regardless of the growth of their mammary glands, etc. and the quantity of daily lactation was measured for comparative study. At the injection, no side-effect could be observed, except for slight pain and reddening at the site.

c) Effect of the fractions

As controls, the mean daily quantity of lactation of 100 puerperae without experience of lactogeogues and free from excessive disturbance of lactation due to complications was computed down to the 6th day of puerperium. The results, as shown in TABLE IV, showed that all the fractions were more or less effective in the stimulation of lactation, though it must be confessed that the tested cases were regrettably few. On the 6th day of puerperium, lactation was increased by 13% after Protein I, by 37% after Protein II and by 12% after Protein III.

4) Chemical properties of the extracts

a) Test-tube tests

The results are shown in TABLE V.

b) Paper chromatography of the amino acids in the proteins

Primary development was effected with butanol plus glacial acetic acid plus water in the ratio of 4 : 1 : 2, secondary development with phenol plus water in

Rabbits after Throwing

Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Injection	▭	▭	▭	▭	▭	▭	▭							
Milk secretion	−	−	−	−	±	+	+	+	+	±	±	−	−	−
Injection	▭	▭	▭	▭	▭	▭	▭							
Milk secretion	−	−	±	+	++	++	++	++	+	+	+	+	±	−
Injection	▭	▭	▭	▭	▭	▭	▭							
Milk secretion	−	±	+	++	++	++	++	+	±	±	+	−	−	−
Injection	▭	▭	▭	▭	▭	▭	▭							
Milk secretion	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE IV. Effect on Lactation in Human Subjects

Extract	Case \ Date	1	2	3	4	5	6
Control	100	13	68	145	221	279	317 (cc)
Protein I	10	13	89	171	216	319	358 (cc)
		100	131	117	98	114	113 (%)
Protein II	10	4	115	206	354	373	434 (cc)
		31	169	142	160	134	137 (%)
Protein III	10	13	57	101	229	296	356 (cc)
		100	84	70	104	106	112 (%)

TABLE V. Results of Test-tube Tests

Tests	Protein I	Protein II	Protein III
Biuret	++	++	++
Molisch	−	−	+
Goldschmiedt	−	−	−
Neuberg-Saneyoshi	−	−	−
Test for phosphorus	−	−	−
Dische for deoxypentose	−	−	−
Millon	+	+	+
Hopkins-Cole	−	−	+
Sakaguchi	+	+	+

the ratio of 4 : 1, temperature : 25±1°C, reagent : 0.1% ninhydrin. The chromatograms of the proteins showed in common spots at the positions corresponding to aspartic and glutamic acids, serine, glycine, threonine, alanine, tyrosine, leucine and/or isoleucine, valine and/or methionine, proline, lysine, histidine and arginine.

Those of Protein III showed spots of phenylalanine and of hexosamine in addition.

c) Electrophoresis

Proteins I, II and III were examined in 0.5 % water solution, phosphate buffer, pH 7.7, I 0.2, 10 mA. All the patterns showed one sharp boundary.

d) Solubility and precipitablity of the preparations

The preparations were insoluble in glacial acetic acid. Their watery solutions precipitated on addition of lead acetate, cupric chloride and trichloroacetic acid and on saturation with ammonium sulfate, but did not on addition of barium-acetate and -chloride.

DISCUSSION

It was Halban[11] (1905) that first discussed placenta in connection with endocrine secretion. He asserted that the growth of the mammary glands in gravidae may be attributed to the agency of placental action, basing himself on clinical observations, and that the milk secretion can be explained as due to a reparation process of the milk glands resulting from the cessation of the stimulation for growth from the placenta, but he did not think of any positive lactogenic function of the placenta. Since then, the literature which is mainly concerned with the placental function in the direction of stimulation of mammary growth and milk secretion has been very copious. Following Halban, Schäfer[12], Frankel[13] and Nelson[14] opined that the placenta had inhibitory action on lactation, while among others Biedl[15], Frank[16], Litt[17], Grüter and Stricker[18] may be mentioned as denying almost any effect of the placenta on it. Kawaida[19], Fellner[20], Hermann[21], Takahashi[22], Miyagawa[23] and Kita[24] belong to the camp that acknowledge some effect on the growth of the mammary glands but do not acknowledge any effect sufficing to cause actual lactation, or going at most to cause secretion of colostrum, in the placenta, while Aschner and Grigoriu[25] asserted that injection with chyle and water or alcohol extraction of the placenta causes growth of the mammary glands and lactation, and Basch[26], Mayer[27], Dohi[28], Taniguchi[29], Ikegami[30], Niklas[31], Hirata[32], Kasamori[33] and Ogawa[34] have arrived to quite similar conclusions.

The present author found that subcutaneous injection of the tested fractions of protein in rabbits that have already lost power of lactation after the last throwing caused resumption of lactation.

Since Riddle *et al.* perfected the method of assaying lactogenic hormones by crop reaction in pigeons, many reports have appeared on the crop reaction after placental substances. Keller[35] found positive crop reaction following transplantation of placenta to pigeons, and says that about one-third of mature placentae contains a lactogenic substance, and this substance is found especially frequently in immature placentae, in hydatidiform moles, and chorionepithelioma.

Ehrhardt succeeded in proving the existence of prolactin in the placenta, in a large content the younger the placenta; he found relatively large quantity of prolactin in the extract from hydatidiform moles, and says that he could obtain a hormone of 10–20 pigeon units from 1 cc of the above substance. Fujii[36] could not find positive crop reaction following intramuscular injection of acidic and alkaline placental extracts of any stage of pregnancy, but could prove prolactin in the placenta of rats only. Ray *et al.*[37] report that a 12 day old placenta of a rat is equivalent to 0.01 mg. of hypophyseal prolaction in the effect of stimulating lactation and to about 2 mg. of it in the effect of stimulating formation of corpora lutea. Averill[38], Astwood[39] and Lyons also acknowledge the effect of rat placenta extract in causing positive crop reaction. Mayer and Canivenc[40] found the stimulating effect on luteal bodies, the growth of mammary glands and even the secretion of milk stimulated after auto-implantation of trophoblast from the placenta of rats, and ascribes stronger effects in these three functions but a weaker effect in stimulating crop reaction to the factor in these cells, in comparison with the respective effects of hypophyseal prolactin. Akasu and Otani[41] and Akasu[42] report that the chorionic gonadotropin extracted from the human placenta was experimentally and clinically confirmed to have a lactogenic action on the mammary glands of multiparae or nulliparae pretreated with the same hormone; they say that this substance is similar to prolactin in its action on the luteal bodies, but differs from it in causing negative crop reaction. Akasu *et al.* found this hormone was very effective in clinical application, Oda[43] admits some lactogenic effect of it, but Matsumoto *et al.*[44] deny a stimulating effect on milk secretion in it. Iida[45] *et al.* report that administration of chorionic gonadotropin to patients with deficient lactation had little effect, and if any, only a transient effect. But Nakamura[46] acknowledged the efficacy of this hormone and recommended it in treating oligogalactic puerperae. More recently, Ito succeeded in extracting a substance effective in causing thickening of the crop mucosa, similar to that after prolactin, from human placentae, especially, mature human placentae, and by isoelectric fractionation, obtained 3 different precipitates of pH 6.5, 6.0 and 5.5, respectively from this substance. Upon making crop reaction with these fraction, he found the pH 5.5 fraction most effective. By improving his method of extraction, he obtained an even more effective fraction by water extraction and ammonium-sulfate fractionating, and reported that this substance was so effective as to cause a conspicuous thickening of the epithelial layer and a marked growth in size and number of lactic fat drops in the crop-milk. This amplification of fat drops was rather perceptible in 48 hrs. after single dosage of the substance already, rose parallel with the increase of the dosage and showed little individual difference, so that this augmentation of fat drops can be useful as index in purifying the prolactin-like substance in the human placenta. His method of extraction is very similar to the one fol-

lowed by the present author, and the effect of the extracts in causing crop reaction is also similar. In the next place, upon clinical application of my extract, the extract proved sometimes very effective but sometimes nearly of no effect in increasing lactation. Such contradictory results, however, may be accepted as rather natural, when we recall that the mechanism of lactation is so complicated and is subject to the influence of many factors concerning mental conditions, development of the mammary glands, the nutrition, etc. of the puerpera. In the next place, we face the question why physiological lactation is inhibited during pregnancy and begins as soon as delivery is over. Akasu[47] says that the gonadotropin in the placenta is all used up in elaborating progesterone therein, so that little is left available for instigating milk production, and also the mammary glands are subject to the strong influence of estrogen-progesterone during pregnancy for stimulating growth of the glands, so that the influence of gonadotropin, if even available, cannot interfere. The effect of my extract also may be partly subject to the same action mechanism.

In the last place, it must be mentioned that some reports on the chemical identity of such placental substances are available. Since Fellner[20] (1913) first reported his success in isolating from the placenta the effective substance acting upon the mammary glands and opined that the substance may be a lipoid, there were reports by Hermann who presumed it to be a cholesterine derivative, by Dohi and Ikegami who saw a lipoid substance in it, by Taniguchi who says that placental phosphatide preferentially stimulates the growth of mammary glands and the placental protein decomposates the secretion of milk. More recently, many authors have presumably identified it with trophoblast hormone, prolactin and such protein hormones. The present author studied the chemical properties of the placental extract and ascertained that the substance consisted in a protein resembling prolactin.

SUMMARY

1. Implantation of placentae of several stages of pregnancy beneath the skin near the crop of pigeons always caused considerable hyperemia, thickening and opacity of the mucous membrane.

2. Placental extract in saline caused negative crop reaction.

3. Placental extracts prepared by Bonsens and White's method, Li's method and Lyon's method always caused negative crop reaction.

4. The three extracts prepared by our method from full-term human placentae with the isoelectric point at pH 5.0, pH 5.8 and pH 6.0 caused always positive crop reaction, 1 mg. of them being equivalent to 1/2, 1 and 2 pigeon units, respectively. Positive reaction also followed intramuscular injection of 20 mg. of pH 5.0 and pH 5.9 fractions and 10 mg. of pH 6.0 fraction.

5. Only the pH 5.8 fraction caused lactation upon injection into the

lactiferous tubules of pseudo-pregnant rabbits.

6. When injected in post-puerperal rabbits with completely stopped lactation, all the fractions caused resumption of lactation.

7. When injected in puerperal women, all the fractions caused the increase of secreted milk over that in the non-treated controls. No side-effect of the administration was perceptible.

8. The chemical properties of the fractions were determined. The results pointed to the inference that it consisted in a prolactin-like protein.

Acknowledgments

In conclusion, the author hastens to express heartly thanks to Prof. K. Kushima for his unfailing kindness in supervising my study and revising this report. My thanks are due also to Dr. H. Yoshizaki for his valuable suggestions and to Dr. S. Matsushima of Teikoku Zoki Pharmacy for his kind assistance in carrying out my experiments.

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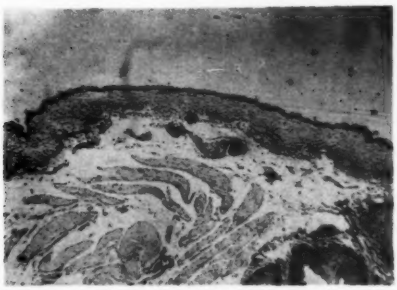

Fig. 1. Section of normal crop sac membrane.

Fig. 2. After intramuscular injection of 20mg. of Protein I. Note the enlarged folds bearing numerous short downgrowths.

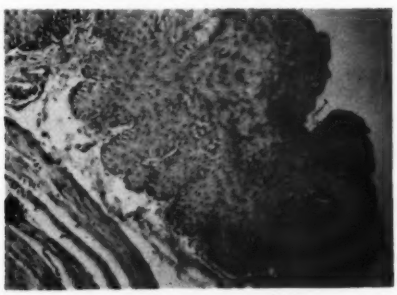

Fig. 3. After intramuscular injection of 10 mg. of Protein II. Note the enlarged folds of thickened mucosa with epithelial downgrowths.

Fig. 4. After intramuscular injection of 10 mg. of Protein III. Note that the enlarged folds bearing characteristic downgrowths of cells are tending to fuse along the adjoining surface.

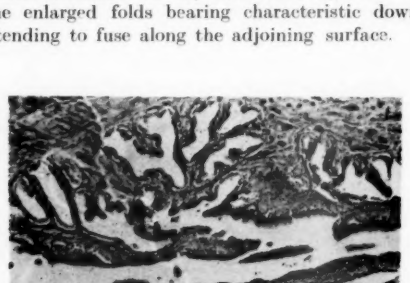

Fig. 5. Mammary gland after injection of Protein I 5 mg. in the milk duct.

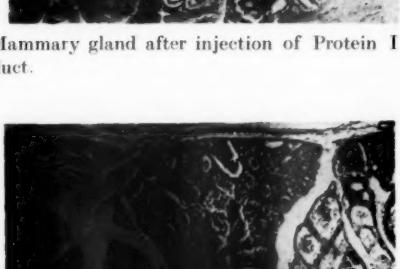

Fig. 6. Mammary gland after injection of Protein II 5 mg. in the milk duct.

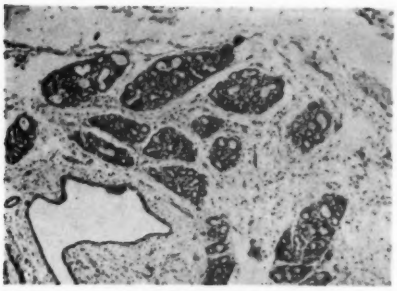

Fig. 7. Mammary gland after injection of Protein III 5 mg. in the milk duct.

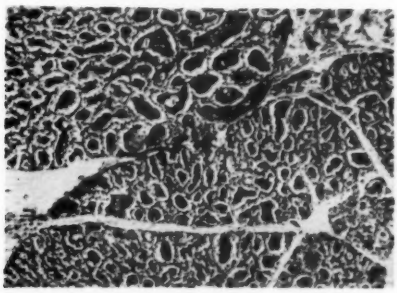

Fig. 8. Mammary gland after throwing injected with Protein I 35 mg.

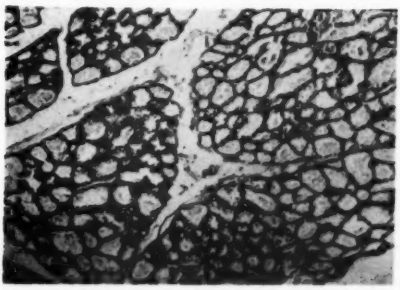

Fig. 9. Mammary gland after throwing injected with Protein II 35 mg.

Fig. 10. Mammary gland after throwing injected with Protein III 35 mg.

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Pantothenic Acid and Age

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In the previous reports[1], the present author has pointed out that the pantothenic acid content in blood decreases with age, particularly in women.

In this study, with the aim of endorsing the above proposition, the author conducted a mass-survey of the blood pantothenic acid content, the serum alkaline phosphatase activity and the total serum cholesterol content in more than 200 residents, males and females, in Ouri Section, Ohira Village, Miyagi Prefecture, a farming community, in Northern Honshu, Japan, and also made an attempt at calculating the physiological age[2)–4)] of the residents. The findings are presented in the following.

SUBJECTS AND METHODS

1. The village section selected for the survey is the model farming community chosen by this laboratory for researches on the effect of countermeasures against hypertension[5)], of which 80 per cent of the residents mainly engage in rice production– a community fairly representative of the communities making up the rice monoculture area in Northern Honshu. The survey and blood sampling required for this study were carried out in February, 1961.

2. The method for estimating the content of pantothenic acid in blood has been described by the present author.[1)]

3. The method for estimating the alkaline phosphatase activity in serum: The Bessey *et al*'s method[6)] was adopted: Using disodium *p*-nitrophenylphosphate as substrate, the quantity in mM of *p*-nitrophenol liberated by *1* liter of serum per hour was determined, to express the activity.

4. The method for estimating the total cholesterol content in serum: The Kiliani reaction method by Bennie Zak[7)] was partly modified by the present author. The details of description are referred to the previous report.[8)]

5. The method for calculating the physiological age: As shown in Table I, according to Amako[2)], Benjamin[3)] and Oshima,[4)] besides the generally used calender age expressing the number of years lived through since one's birth,

石 黒 弘 三

TABLE I. The Calculation of the Physiological Age

The Physiological Age=The Outward Looks+The Functional Fitness

The Outward Looks
- Hairs (Gray hair, Alopecia, ...)
- Eyes (Ring of The Cornea, Enophthalmos, ...)
- Teeth (Untooth, Ramus of the Mandible, ...)
- Skin (Turgor of The Skin, Pigmentary Deposit, ...)

The Functional Fitness
- Visial Power (Presbyopia, ...)
- Auditory Power
- Vital Capacity
- Cardiopulmonary Quotient
- Frequency of Urination in Winter
- Urobilin Reaction

The 10 items, above mentioned, 4 concerning the outward looks and 6 concerning the functional fitness, are selected. The marks of bad are given (2), unsatisfactory (1) and good (0) for all the items.

Fig. 1. The sketch of the section for survey.

we should pay due consideration to one's physiological age of mainly biological significance, taking the outward appearance and the functional ability of the subject. The present author, after full consideration of the opinions of these authors, selected 10 appropriate items, 4 concerning the outward appearance and 6 concerning the functional fitness, and gave the marks of bad (2), unsatisfactory (1) and good (0) for all the items, to compute the physiological age.

For marking the items, specialists consulted for evaluating the outward apparance, the visual and the auditory powers were elicited by inquiry, the vital capacity below 1,500 cc was marked (2), between 1,500 and 2,500 cc (1) and above 2,500 cc (0), the cardiopulmonary quotient below 7 was rated (2), between 8 and 10 was rated (1) and above 11 was marked (0), and the frequency of urination in winter night of more than 4 times was rated (2), 2–3 times (1) and 0–1 time (0), positive urobilin-reaction in urine was given (2) and negative cases (0).

RESULTS

A) In Table II and Fig. 2 are shown the mean content of pantothenic acid in blood of the subjects grouped by age classes, and in Table III, Fig. 3 and Table. IV, Fig. 4, the serum alkaline phosphatase activity and the total serum cholesterol

Table II. The Mean Values of Pantothenic Acid Content in Blood Grouped by Age Classes

Age Class	No.	Male	No.	Female
yrs.		γ/dl		γ/dl
30–39	26	89.9±12.2	22	106.0±27.2
40–49	22	91.8±14.6	40	100.5±10.8
50–59	25	88.0±11.4	16	83.5±21.6
60–	18	73.7±26.3	19	74.2±11.3

The numerals denote the mean ± 95 per cent confidence limit.

Table III. The Mean Values of Alkaline Phosphatase Activity in Serum Grouped by Age Classes

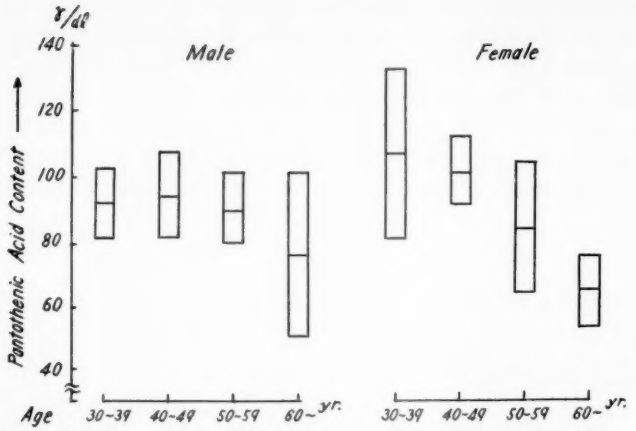
Age class	No.	Male	No.	Female
yrs.		n. ph. unit*		n. ph. unit*
30–39	19	2.63±0.33	23	2.36±0.39
40–49	18	2.60±0.34	35	2.14±0.27
50–59	21	3.05±0.28	12	2.64±0.45
60–	14	3.05±0.52	18	2.96±0.31

The numerals denote the mean ± 95 per cent confidence limit.
* The n. ph. unit expresses nitrophenol unit.

Table IV. The Mean Values of Total Cholesterol Content in Serum Grouped by Age Classes

Age class	No.	Male	No.	Female
yrs.		mg/dl		mg/dl
30–39	24	151.0±12.6	26	160.6±14.1
40–49	18	171.5±14.7	39	170.0± 7.2
50–59	23	177.4±24.4	15	195.6±23.5
60–	17	178.8±15.9	19	178.2±17.3

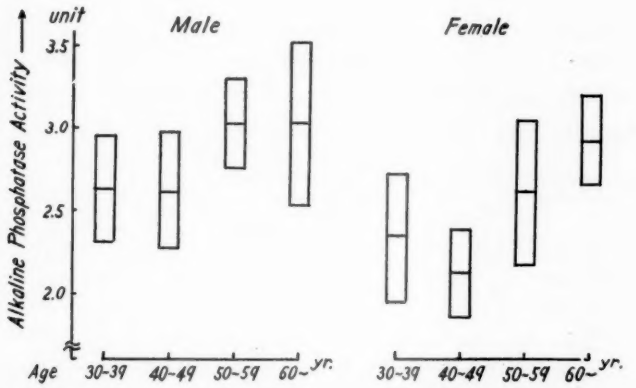
The numerals denote the mean ± 95 per cent confidence limit.



subjects No. (26) (22) (25) (18) (22) (40) (16) (19)

Upper and lower limits denote the 95 per cent confidence limit of mean.

Fig. 2. The mean values of pantothenic acid content in blood by age classes.



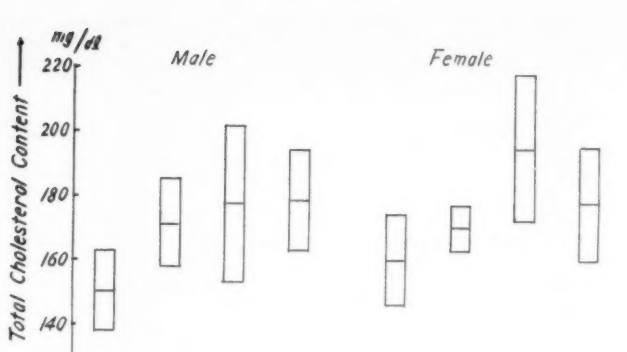
subjects No. (19) (18) (21) (14) (23) (35) (12) (18)

Upper and lower limits denote the 95 per cent confidence limit of mean.

Fig. 3. The mean values of alkaline phosphatase activity in serum by age classes.

content in averages per age classes.

As shown in Table II and Fig. 2, the distribution of blood pantothenic acid content is different in males from that in females. Among males, the content remains on nearly the same level from the thirtieth through the fiftieth and begins to fall off only in the sixtieth. In the age classes above 60, the dispersion of the values rises to 60γ/dl, as shown in Fig. 5, indicating the wide individual



subjects No. (24) (18) (23) (17) (26) (39) (15) (19)

Upper and lower limits denote the 95 per cent confidence limit of mean.

Fig. 4. The mean values of total cholesterol content in serum by age classes.

TABLE V. The Distribution of the Physiological Age (Ph. A.) Grouped by Age Classes

	Ph. A. \ Age	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70- yr.	Total
Male	0— 1	4	1								5
	2— 3	8	9	1	2						20
	4— 5	2	6	7	2	1					18
	6— 7			2	5	6	1	1			15
	8— 9			2	1	10	1	4	1		19
	10—11					1	2	1	5	3	12
	12—13					2	3	1	2	2	10
	14—15							1		3	4
	16—17							1		1	2
Total		14	16	12	10	20	7	9	8	9	105
Female	0— 1	3									3
	2— 3	4	6	2							12
	4— 5	5	5	8	1	1					20
	6— 7	1	5	9	7	2	1				25
	8— 9			4	8	3	1	2	1		19
	10—11				6	8	2	2	2	4	24
	12—13						2	3	4	4	13
	14—15							1		1	2
	16—17							1			1
Total		13	16	23	22	14	6	9	7	9	119

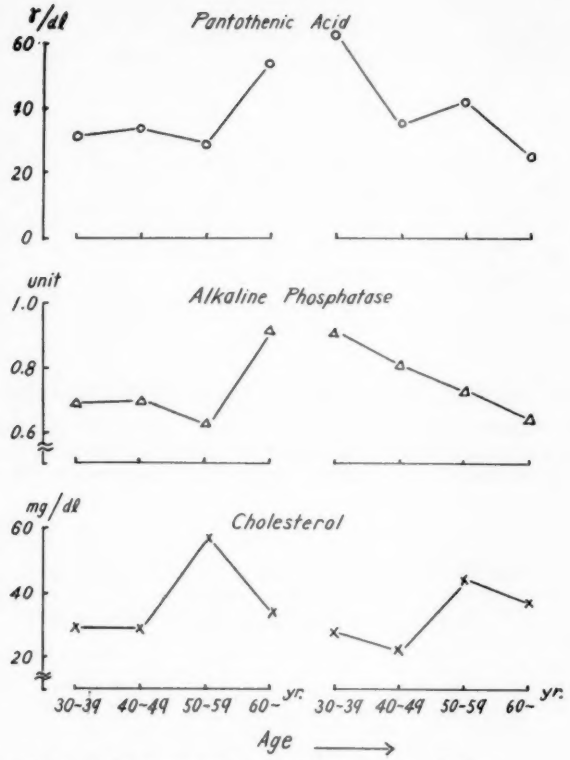


Fig. 5. The dispersion of the values by age classes

difference here. On the other hand, among females, the blood pantothenic acid content tends to come down steadily with age, showing the significant differences in the mean values of the thirtieth and the fortieth from that of the sixtieth respectively ($p<0.05$). The dispersion of the values is rather wider in the younger age classes, being reduced to only 20γ/dl in the age above 60.

Stern[9] and Vermehren[10] have already reported that the activity of alkaline phosphatase in serum is high in infants, and abates gradually in children and adults, and Fujita,[11] Robertson[12] and other authors have pointed out that this activity rises again in old age; Sato[13] of this laboratory has also obtained similar results. In the present study too, the activity was found higher in the age classes above 50 than the younger among both sexes, especially the mean value among females of more than 60 years being significantly higher than that in the fortieth.

The dispersion of values was wider in the age above 60 among males and in

TABLE VI. The Distribution of the Age by Outward Looks Grouped by Age Classes

	Ph. A. \ Age	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70- yrs.	Total
Male	0	6									6
	1	4	5								9
	2	1	6	5	2	1					15
	3	2	4	2	3	5		2			18
	4	1		4	5	4	1				15
	5		1	1		6	3	3			14
	6					4	2	2	3	2	13
	7						1	1	4	1	7
	8							1	1	6	8
Total		14	16	12	10	20	7	9	8	9	105
Female	0	4		1							5
	1	6	2	1							9
	2	3	9	3	2	1					18
	3		3	10	3						16
	4		2	8	9	4	2				25
	5				8	5	1			2	16
	6					4	2	4	2	2	14
	7						1	4	2	1	8
	8							1	3	4	8
Total		13	16	23	22	14	6	9	7	9	119

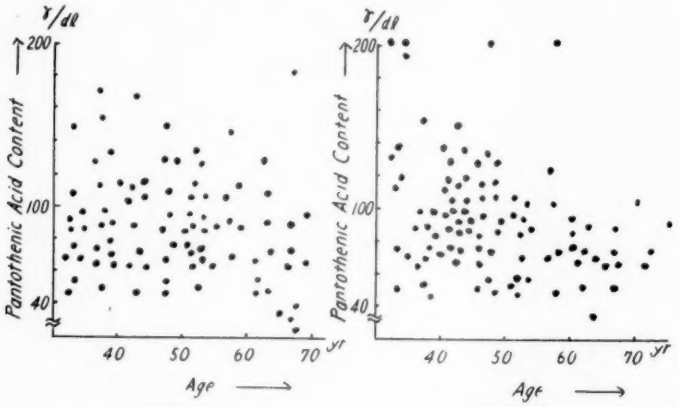
TABLE VII. The Distribution of the Age by Fitness Grouped by Age Classes

	Ph. A. \ Age	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70- yrs.	Total
Male	0— 1	9	13	5	4	1		1			33
	2— 3	5	2	4	4	7	2	1	3		28
	4— 5		1	2	2	10	1	2	4	5	27
	6— 7			1		2	4	4	1	3	15
	8— 9							1		1	2
	10—11										
	12—										
Total		14	16	12	10	20	7	9	8	9	105
Female	0— 1	4	5	3	1	1					14
	2— 3	5	9	14	7	3	1	3	1	1	44
	4— 5	4	2	6	9	8	3	3	5	4	44
	6— 7				5	2	2	2	1	4	16
	8— 9							1			1
	10—11										
	12—										
Total		13	16	23	22	14	6	9	7	9	119

the younger age among females, as that of pantothenic acid content above.

The total cholesterol content in serum also showed a tendency of rising with advance of age, but in no case was found the significant difference between two age classes.

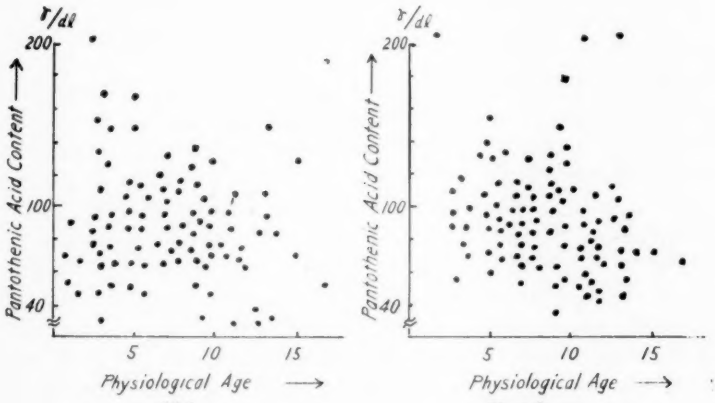
B) In Tables V, VI and VII are shown the distribution of physiological age, age by outward appearance and by functional fitness as itemized by calender age classes. These figures seem to suggest that among females in the fortieth



Male N=91 r=−0.152 P>0.10

Female N=97 r=−0.284 P<0.01

Fig. 6. The correlation between pantothenic acid content in blood and age.

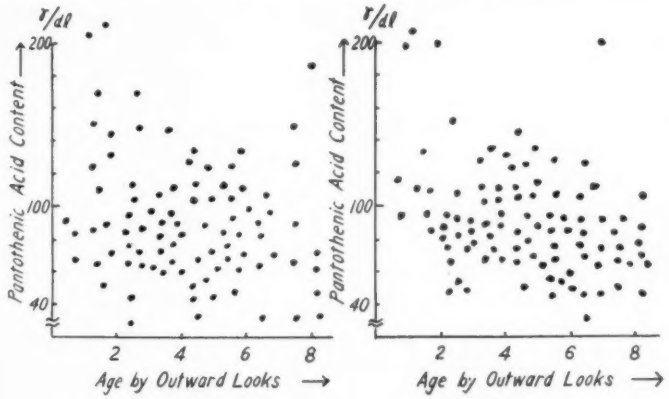


Male N=90 r=−0.011 P>0.01

Female N=97 r=−0.225 P<0.05

Fig. 7. The correlation between pantothenic acid content in blood and physiological age.

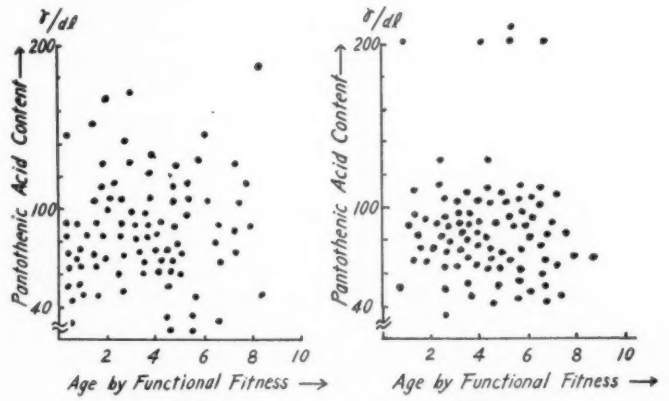
there are many who look older than their age. On the other hand, the distribution of age by functional fitness was not so definitely traceable in the age classes as that of age by outward appearance, that is to say that the 6 items concerning the functional fitness selected by the author in this survey were inadequate to some degree in evaluating the functional age. This point will be discussed later on in the paragraph on the relationship between the blood panto-



Male N=90 r=−0.150 P>0.10

Female N=97 r=−0.307 P<0.01

Fig. 8. The correlation between pantothenic acid content in blood and age by outward looks.



Male N=90 r=0.071 P>0.10

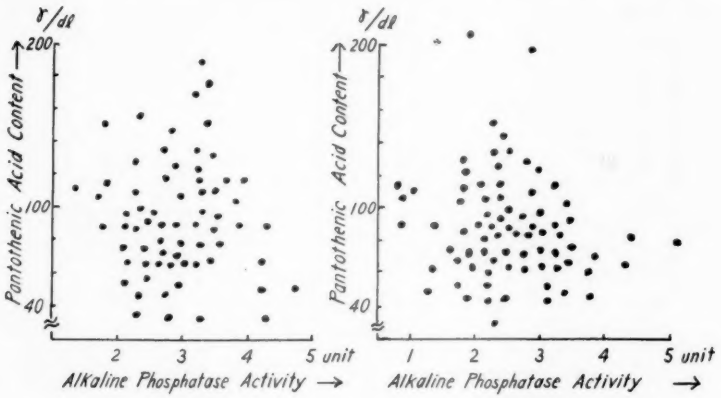
Female N=97 r=−0.034 P>0.10

Fig. 9. The correlation between pantothenic acid content in blood and age by functional fitness.

thenic acid content and the physiological age.

C) Between the pantothenic acid content in blood and the age was established a negative correlation, as shown in Fig. 6, too low to be significant among males, but significant at the level of significance of 1 per cent among females.

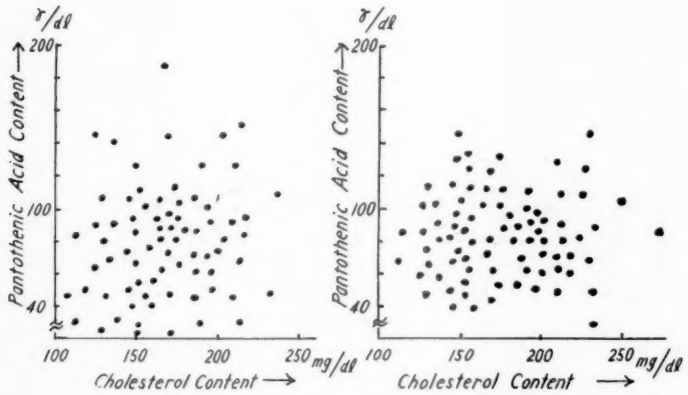
Next, as shown in Figs. 7, 8 and 9, significant negative correlation was found between the blood pantothenic acid content and the physiological age ($P<0.05$)



Male N=65 r=−0.116 P>0.10

Female N=78 r=−0.214 P<0.10

Fig. 10. The correlation between pantothenic acid content in blood and alkaline phosphatase activity in serum.



Male N=74 r=−0.083 P>0.10

Female N=88 r=−0.072 P>0.10

Fig. 11. The correlation between pantothenic acid content in blood and total cholesterol content in serum.

and between those content and the age by outward looks ($P<0.01$) among females only, the age by functional fitness being not significantly correlated with the acid content in either sex.

Figs. 10 and 11 show the correlation between the pantothenic acid content in blood on the one hand and the activity of alkaline phosphatase and the total content of cholesterol in serum on the other. We see that only a barely negative significant correlation ($r=-0.214$, $P<0.10$) prevails between the pantothenic acid content in blood and the alkaline phosphatase activity in serum among females.

DISCUSSION

The community treated in this study, as stated at the outset, is a section in a farming village in Northern Honshu, mainly producing rice and little else and accepted as poor in alimentary means and other living amenities. However recent development seems to have led to betterment of living conditions, slowly but steadily. In August 1950, the present author has conducted a survey of pantothenic acid content in blood in 78 of the inhabitants of the section, and found that the content fell off with age, particularly among women, and in the present survey with more than double subjects, the results were the same. In general, the concentration in blood of B-group vitamins is said to be subject to show wide individual differences, and pantothenic acid is not exempt from this general rule, either. This can be readily seen in Fig. 5. In this connection, it must be noted well that the dispersion of the values shows an inverse relation among males and females. In the age classes above 60 years, the mean values of pantothenic acid in blood among males and females stand on the similar levels of 73.7 and 74.2γ/dl. However the dispersion of the values among females of this age is limited in a far smaller range than among males, that is to say, the blood pantothenic acid content seems to be low among old women "without much exception."

In Japan, Watanabe *et al.*[14] have described a detailed report on the activity of alkaline phosphatase in serum. The increase of this enzyme activity in old age seems to suggest that, besides playing parts in a processes of osteogenesis, digestion and absorption of lipids and glycides, tissue-regenerations and such synthetic functions, this enzyme also partakes in some process of a phenomenon "older aging". In fact the present author *et al.*[15] have already found a close positive correlation between the activity of this enzyme in serum and blood pressure, both systolic and diastolic.

Many observed facts [16][17] seem to indicate that the total cholesterol content in serum is related with the quantity and quality of lipids in foods, but in our study[8] on the cholesterol content in serum of inhabitants of some farming and fishing villages in Northern Honshu, we have found the content ranged between 140 and 190 mg/dl and showed higher values among the farming inhabitants

who consume less lipids than in the inhabitants of the fishing village where lipids are consumed more abundantly. This findings suggest that the serum cholesterol content is under the influence not only lipids in diet but also of other food stuffs and climate (the fishing village we surveyed are known to have warmer winters than the inland areas). The results of our trophic survey in the farming villagers are shown in Table VIII.

On the seasonal change of serum cholesterol, Watanabe *et al*[18] have also described a compendious reports. They ascribe the cause of decrease of the serum cholesterol content in summer, besides to the decrease of lipids taken in, to a lowered elaboration of cholesterol due weakened function of the liver and to a tendency of hightened function of the thyroid gland and the adrenal cortex in the hot season. The present author twice conducted mass-estimations of serum cholesterol in the same area, once in August, 1960 and again in February, 1961, and found the measured content to be low in summer and high in winter (Fig. 12). The slow rise of serum cholesterol content with advanced age, as already pointed out by some past authors,[19][20] has been also confirmed in this survey. Guehring *et al.*[21] have pointed out that pantothenic acid plays a part in the process of cholesterol metabolism in the liver, and many recent authors have supported this proposition, but the author could find no direct correlation between the pantothenic acid content in blood and the total cholesterol content in serum (Fig. 11).

TABLE VIII. Daily Intake of Nutrients and Foods Characterized Monoriceculture Area in Northern Honshu, Japan

The Intake of Nutrients

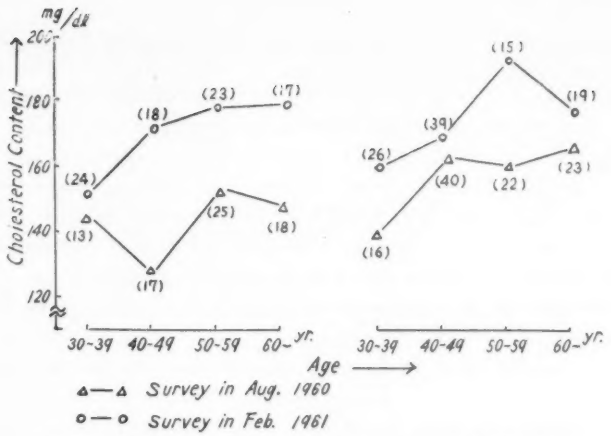
	Cal.	Protein	Animal Protein	Carbohydrate	Fat	Ca	P	Fe
	cal.	g	g	g	g	mg	mg	mg
Summer	2347±60	69.5±2.3	37.4±5.3	461±12	32.0±2.0	456±30	1579±50	15.6±0.8
Autumn	2442±84	77.9±3.9	28.7±2.9	487±16	25.0±1.6	420±26	1709±69	14.3±0.9

	Vit. A	Vit. B_1	Vit. B_2	Vit. C
	I.U.	mg	mg	mg
Summer	1183±330	1.25±0.04	0.87±0.04	124±11
Autumn	1667±278	1.25±0.05	0.87±0.05	112±10

The Intake of Foods

	Rice	Barley	Potato	Bean	Animal Foods	Vegetable	
						whole	(green & yellow)
	g	g	g	g	g	g	g
Summer	420±14	100±12	83.1± 9.7	53.6±4.4	168±21	440±34	81.8±14.9
Autumn	492±21	63± 9.2	78.9±12.1	69.9±6.1	168±20	470±44	49.5± 7.9

The numerals denote the mean ± standard error.
30 homes are selected for the survey.



The numerals in parentheses denote numbers of subjects.

Fig. 12. The comparison of mean total cholesterol content in summer and winter.

For determining the physiological age, Amako[2] has adopted 8 items concerning the outward looks and 9 items concerning the functional fitness as measures. Benjamin[3] selected 20 items and Oshima[4] 25 items for this purpose. The present author had to reduce the items to 10 for convenience in mass-examination (Table I). The inadequacy of these items, as stated above, it seems, has regrettably vitiated my results concerning the distribution of the functional age, at least. This inadequacy was also reflected on the correlation between the functional age and the blood pantothenic acid content, so that whereas the age by outward looks was negatively correlated very closely with the acid content among women ($P<0.01$), no significant correlation could be established between the functional age and the content. Or, it might be that the pantothenic acid content in blood is in closer connection with the outward looks, via the functional fitness. However the author feels rather diffident for daring such an interpretation. A significant negative correlation at the level of significance below 10 per cent was observed between the activity of alkaline phosphatase in serum and the content of pantothenic acid in blood among females, but it is not clear whether this correlation is indirect (pantothenic acid content→age→alkaline phosphatase activity) or direct (pantothenic acid→alkaline phosphatase).

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SUMMARY

1) A mass-survey of the pantothenic acid content in blood, the alkaline phosphatase activity and the total cholesterol content in serum was undertaken in an agrarian area with some 200 of the inhabitants over 30 years of age as subjects. The physiological age of the subjects was also estimated.

2) The mean pantothenic acid content in blood remained nearly stationary from 30 to 60 years but fell off gradually thereafter among males, while among females, the content went on declining with age, the mean value in the age classes of above 60 years showing significant drop at the level of significance below 5 per cent from that in the age classes of 30–39 and 40–49 years.

3) The activity of alkaline phosphatase in serum was found rising with advance of age above 50 years among both sexes, the mean value of above 60 years among females being significantly higher than that of 40–49 years among females.

4) The total cholesterol content in serum tended to increase with age, but not significant.

5) The pantothenic acid content among females was found correlated negatively with their calender age, physiological age and age estimated by outward looks, at the level of significance below 1, 5 and 1 per cent, respectively.

6) A significant negative correlation could be found between the activity of alkaline phosphatase in serum and the pantothenic acid content in blood, at the level of below 10 per cent.

7) No significant correlation was observed between the total cholesterol content in serum and the pantothenic acid content in blood.

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Urinary Excretion of Catecholamines and Their Metabolites in Normotensive and Hypertensive Subjects

By

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INTRODUCTION

Catecholamines are considered to be neurohumoral transmitter of the sympatho-adrenal system and to be powerful pressor substances naturally found in the body. And the urinary excretion of noradrenaline has been shown to vary quite sensitively with changes in the sympathetic system. On the other hand, it has been proved that paroxysmal or sustained hypertension in the cases of pheochromocytoma is caused by a large quantity of catecholamines released from chromaffine cell tumor. Thus, it is a very interesting problem to evaluate the role of sympatho-adrenal system in the pathogenesis of essential hypertension.

We have been studying this problem for the past several years, and no significant difference in urinary excretion of catecholamines was found between normal subjects and patients with essential hypertension.[1] Accordingly, we concluded that essential hypertension does not result from an excess of catecholamines.

Recently, the metabolic pathway of catecholamines has been clarified by several investigators, who also described satisfactory methods for the quantitative study of these metabolites.[2-4] And a colorimetric method for the determination of urinary metadrenaline (M), normetadrenaline (NM) and vanillylmandelic acid (VMA) could be established in our laboratory.

The present study was undertaken in an effort to confirm the previous data from the new point of view, i.e. catecholamine metabolism.

EXPERIMENTS AND RESULTS

1) *Methoxycatecholamines in urine*

3-methoxy derivatives of adrenaline and noradrenaline in urine were

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measured by the method which was reported previously in a separate paper[5]. This method was based on high voltage paper electrophoretic separation of these compounds in the urinary extract. In brief, acidified urine was hydrolysed and extracted with ethylacetate. The extract was evaporated to dryness, the residue was dissolved in a small amount of methylalcohol, and then subjected to high voltage paper electrophoresis. After separation of M and NM, they were converted into colored complex with diazotized *p*-nitroaniline and then eluted from the paper with alkaline methanol. Estimation was made spectrophotometrically.

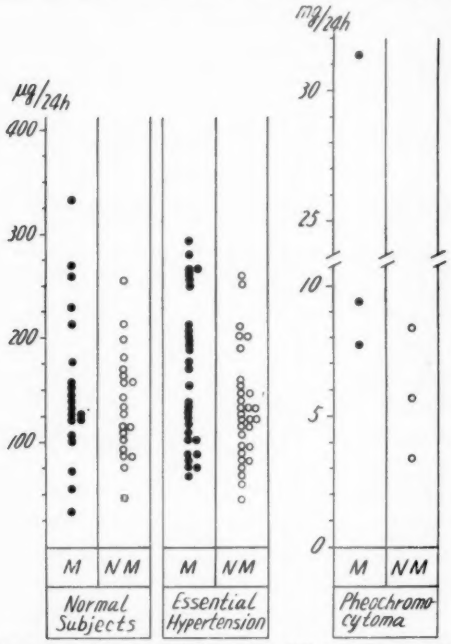

Fig. 1. Urinary excretion of metadrenaline (M) and normetadrenaline (NM).

The urinary output of M and NM was analysed in 20 normal and 30 hypertensive subjects. As shown in Fig. 1, no significant difference in M and NM excretion was demonstrated between normal and hypertensive subjects. The urinary excretion of M in normal subjects ranged from 30 to 330 μg. per 24 hours with an average of 138 μg.; that for the patients with essential hypertension ranged from 55 to 298 μg. per 24 hours with an average of 142 μg. The excretion of NM in normal subjects was 43, 250, 119 and in hypertensives 40, 256, 130 μg. respectively. On the other hand, in three cases of pheochromocytoma M ranged from 7.7 to 31.4 mg. and NM from 3.3 to 8.3 mg. per 24 hours.

2) *Vanillylmandelic Acid (VMA) in Urine*

VMA was measured essentially by high voltage paper electrophoretic method of von Studnitz,[6] with several minor alterations. We used pH 3.6 buffer solution (pyridine-acetic acid-water, 1 : 10 : 89) instead of pH 6.0 buffer (10 : 1 : 89) of von Studnitz.

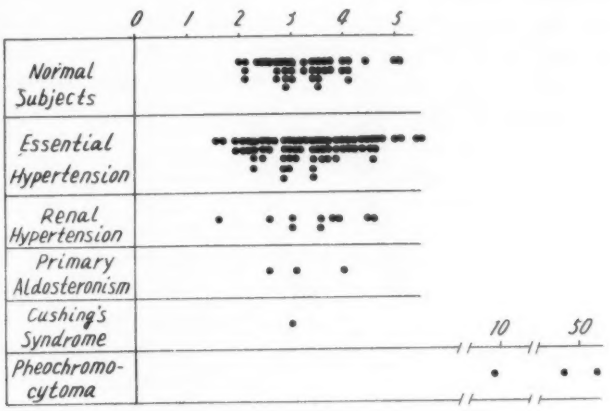

Fig. 2. Urinary excretion of vanillylmandelic acid (VMA).

The concentration of VMA in urine ranged from 2.0 to 5.2 mg. per 24 hours with a mean value of 3.20 mg. in 40 normal individuals and from 1.6 to 5.6 mg. per 24 hours with a mean value of 3.35 mg. in 68 patients with essential hypertension as illustrated in Fig. 2. In addition, we have had an opportunity to examine 10 cases of renal hypertension, 3 cases of primary aldosteronism and one case of Cushing's syndrome while this work was in progress. As shown in Fig. 2, urinary excretion of VMA in these cases was also within normal range. Urine from three cases of pheochromocytoma, however, contained 2 to 10 times as much VMA as normal values, viz. 9.6, 47.3 and 54.0 mg. per 24 hours.

3) *Urinary excretion ratio of catecholamines and their metabolites*

The ratio of catecholamines/methoxycatecholamines/VMA was calculated from the data obtained by estimation of them in 24 hour urine samples which were collected in bottle containing 10~15 ml. of conc. HCl. Urine samples from 20 cases of normal subjects, 20 cases of essential hypertension and three cases of pheochromocytoma were analysed. Free catecholamines were determined by the method of von Euler and Floding.[7] Although adrenaline (A) and noradrenaline (NA) were measured separately by this method, the values of catecholamines cited here are for combined A plus NA. The respective methoxy-derivatives of A and NA were also given in the same manner. As shown in Fig.

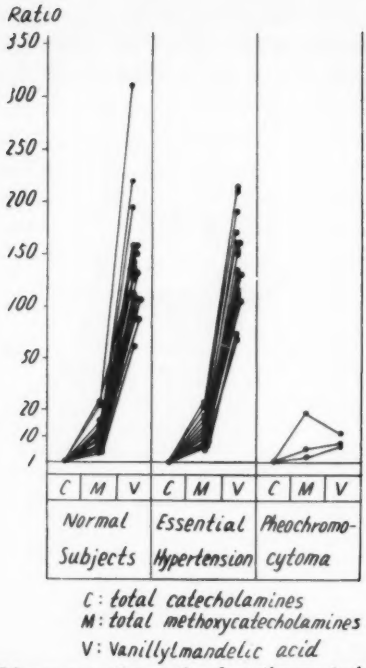

Fig. 3. Urinary excretion ratio of methoxycatecholamines and vanillylmandelic acid to catecholamines.

3, in the normal subjects the ratio was 1 : 10 : 138, in the patients with essential hypertension it was 1 : 12 : 136 on an average. No significant difference was found in this ratio between normotensives and hypertensives. On the other hand, in the cases of pheochromocytoma the ratio was 1 : 9 : 8 on an average.

DISCUSSION

Recently, several authors reported that the estimation of increased amounts of catecholamines and their metabolites is useful in establishing the diagnosis of pheochromocytoma. According to Gitlow *et al*[8]., who analysed urinary excretion of the final product of catecholamines, VMA, there was no overlapping of its excretion between patients with pheochromocytoma and with essential hypertension. In addition, they also reported that the same amount of VMA excretion was observed in urine from patients with essential hypertension as from normal subjects. LaBrosse *et al.*[9] first described the marked elevation of 3-methoxy derivatives of catecholamines in urine from patient with pheochromocytoma and mentioned the usefulness of determination of these metabolites

in making the diagnosis of it. Crout *et al.*[10] estimated the total metadrenaline excretion in urine and also observed no statistically significant difference between 91 cases of essential hypertension and 31 cases of normal controls. Our data well agree with their results.

In previous report,[1] we have observed that there is no significant difference in urinary output of noradrenaline between normotensives and hypertensives, and concluded that hypertension except pheochromocytoma is not caused by an excess of noradrenaline. Findings in this present study from the point of catecholamine metabolism indicate that the previous observation is correct and confirm that conclusion.

In addition, these results were further supported by the findings in calculation of the ratio ; catecholamines/methoxycatecholamines/VMA. As mentioned in the section of experiment, no significant difference in this ratio was found between normal subjects and patients with essential hypertension. Mendlowitz *et al.*[11] suggested a deficit of enzyme which inactivates noradrenaline in patients with essential hypertension. Moreover, they also mentioned that the problem whether or not a hereditary deficit of the enzyme, O-methyltransferase, is the cause of essential hypertension can be established only by appropriate chemical studies. If their thought is correct, an increment of catecholamines or a decrement of methoxycatecholamines and VMA in urine from patients with essential hypertension as compared with normal subjects must be observed, when the current knowledge of its metabolic pathway does not mislead us. But our data cannot support their idea.

There was, however, a significant difference in the excretion of catecholamines and their metabolites or in the excretion ratio of them between patients with pheochromocytoma and normal subjects or patients with essential hypertension. This may be accounted for by the difference in the quantity and metabolism of catecholamines between the two groups ; in the cases of pheochromocytoma large amounts of pressor amines are secreted from the tumor directly into the blood stream and then metabolized as circulating amines in various tissues, while in the other groups they are released at the nerve endings and degraded as tissue amines immediately at the site. According to Goodall *et al.*,[4] who analysed the excretion pattern of infused catecholamines, the infusion of A and NA resulted in an increased excretion of them which ranged from 3 to 5 per cent, of methoxycatecholamines ranging from 40 to 50 per cent and of VMA ranging from 35 to 40 per cent of the doses administered. Thus, the urinary excretion ratio is calculated roughly as 1 : 10 : 10 from their data. This ratio also well agrees with our data obtained from the cases of pheochromocytoma. In other words, hypertension in pheochromocytoma is similar to the state of elevation of blood pressure due to the infusion of an excess of catecholamines. On the other hand, Crout *et al.*[10] studied the catecholamines and their metabolites in urine

from 23 cases of pheochromocytoma and observed two types of patterns of their excretion. In one type of them the increase of excretion of catecholamines was the highest and in the second type of them the increase of excretion of its metabolites was the largest. Our three patients with pheochromocytoma, thus, belong to the former type.

In both normal and hypertensive subjects except pheochromocytoma, however, this ratio was 1 : 10 : 140 on an average. This ratio may easily be understood because in these cases almost all catecholamines are released at the nerve endings and metabolism of them occurs chiefly in the tissues prior to their release into circulation. In any case, it is a more reliable and helpful index in establishing the diagnosis of pheochromocytoma to determine not only catecholamines but also methoxycatecholamines and VMA, and moreover to evaluate this ratio than to determine the respective values of catecholamines, methoxycatecholamines or VMA separately.

The question still remains, however, whether or not essential hypertension is due to hyperresponsiveness of arterioles to a given dose of pressor amines. This cannot be answered in this present study and further investigation will be awaited.

SUMMARY

The urinary excretion of catecholamines and their metabolites was determined and the ratio among their values was calculated in normal and hypertensive subjects.

No significant difference in the excretion of methoxycatecholamines and vanillylmandelic acid was found between normal subjects and patients with essential hypertension.

The ratio of catecholamines/methoxycatecholamines/vanillylmandelic acid proved to be almost the same between them.

From these results, we discussed the problem of the pathogenesis of essential hypertension and could not support the hypothesis that an overactivity of sympatho-adrenal system or an abnormality in the enzymatic system of catecholamine inactivation is present in essential hypertension. In addition, we discussed the value of determination of catecholamines and their metabolites in making the diagnosis of pheochromocytoma.

Acknowledgment

We are indebted to Prof. Dr. T. Torikai for his continuous leadership throughout this study. And we are grateful to Dr. M. D. Armstrong for gifts of VMA, to Prof. S. Seto and Dr. Y. Ikegami, Chemical Research Institute of Non-aqueous Solutions, Tohoku University, for the synthesis of VMA at our request, and to Dr. J. Axelrod for giving us metadrenaline and normetadrenaline.

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Precision Anesthesia Using Two Volatile Anesthetic Agents—Apparatus and Method

By

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Although it is still a matter of controversy, a combined use of various anesthetic agents with different pharmacological properties, so-called balanced anesthesia, has become more popular during the last decade. A reduction of dosis or concentration of the drugs required for the maintenance of surgical anesthesia and a low incidence of undesirable side effects may be highly advantageous. On the other hand, however, a less simplicity in anesthesia and a relatively low controllability may be disadvantages in this type of anesthesia.

A simultaneous administration of two or more different volatile anesthetic agents, like fluothane-ether or chloroform-ether might offer quite interesting problems to anesthesiologists if these are given under precise and dynamic control of vapor concentration and mixing ratio of each agent. However, such a method has never been reported yet in clinical practice, probably because of technical difficulties.

Special devices have been on trial in our laboratory and some of them are going to be commercialized in this country. The methods and devices are described and discussed in details in the present paper.

The principle is a use of two or more copper kettle vaporizers, each filled with different volatile anesthetic agents which are vaporized by oxygen inflow separately and the vapors are conducted to a mixing chamber in parallel through a selector with a piping system.

A new anesthetic apparatus designed forso-called precision anesthesia using two volatile anesthetic agents— a new double copper kettle machine

1. Structure

The apparatus designed by the authors is shown in Photo 1 and is illustrated schematically in Fig. 1. The apparatus is provided with two copper kettle

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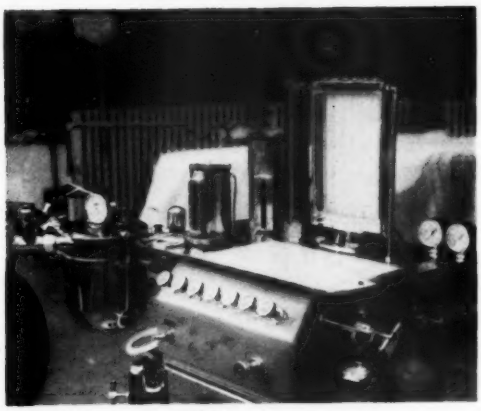

Photo. 1.

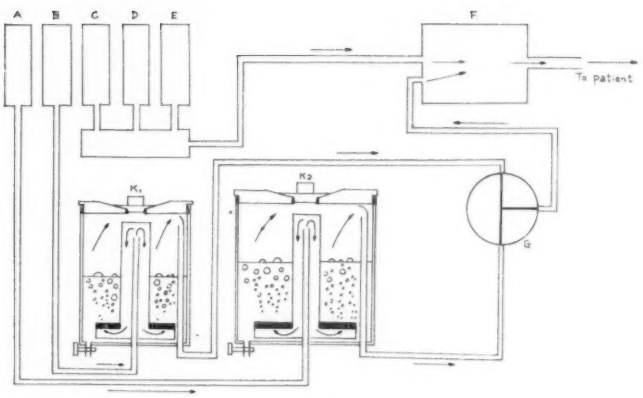

Fig. 1.

vaporizers (K_1 and K_2), each equipped with a flowmeter for oxygen inflow to the vaporizer (A and B), a selector (G), a mixing chamber (F), flowmeters for bypass-oxygen, -nitrous oxide and -cyclopropane (C. D and E) and a respiratory circle (patent pending 35–45089).

If one puts different volatile anesthetics into each vaporizer, for instance fluothane to the first vaporizer (K_1) and ether to the second one (K_2), the respective concentrations of fluothane and ether and the mixing ratio of fluothane /ether in a gas mixture supplied to the inhaler can be regulated easily, accurately

and expediently by simply controlling the diluent or bypass flow (F_d) and the oxygen inflow to the copper kettle 1 and 2(F_{k_1} and F_{k_2}).

If only one agent is desired to be administered, the selector should be switched to such a direction as the anesthetic vapor desired can pass through the selector towards the mixing chamber.

In our first trial product one vaporizer was made a little larger than the other. Basis for this difference in size is discussed later in this paper.

2. Performance characteristics (theoretical)

As previously described by Morris[1] and Feldman and Morris[2] oxygen which flows through a liquid anesthetic is finely dispersed by passing through a sintered bronze disk (porex), and the magnitude of tiny bubbles leads to maximal vaporization efficiency by providing a greatly increased surface for the liquid-gas interface. In other words, the oxygen passed through the copper kettle vaporizer containing a volatile anesthetic agent is to be saturated instantaneously by the anesthetic vapor at the particular working temperature.

Therefore, the performance characteristics of the new double copper kettle machine are well expressed with the formulas described below.

$$A=\frac{F_{k_1}\times P_1}{(760-P_1)\left\{F_d+F_{k_1}\left(1+\frac{P_1}{760-P_1}\right)+F_{k_2}\left(1+\frac{P_2}{760-P_2}\right)\right\}}\times 100\% \tag{1}$$

$$B=\frac{F_{k_2}\times P_2}{(760-P_2)\left\{F_d+F_{k_1}\left(1+\frac{P_1}{760-P_1}\right)+F_{k_2}\left(1+\frac{P_2}{760-P_2}\right)\right\}}\times 100\% \tag{2}$$

$$F_{k_1}=\frac{A}{B}F_{k_2}\times\frac{P_2(760-P_1)}{P_1(760-P_2)} \tag{3}$$

$$F_{k_2}=\frac{B}{A}F_{k_1}\times\frac{P_1(760-P_2)}{P_2(760-P_1)} \tag{4}$$

The marks used in these functions are defined as follows:

F_d(cc. /min.): Diluent or bypass flow. Sum of gas flows which directly go to the mixing chamber.

F_{k_1}(cc. /min.): Kettle flow 1.

F_{k_2}(cc./min.): Kettle flow 2.

Both are oxygen inflow to the vaporizer K_1 and K_2.

P_1(mmHg): Vapor pressure of the volatile anesthetic agent (a) contained in the copper kettle 1.

P_2(mmHg): Vapor pressure of the volatile anesthetic agent (b) contained in the

copper kettle 2.

A(% v/v): Vapor concentration of the anesthetic agent (a) in the whole gas mixture supplied to the inhaler.

B(% v/v): Vapor concentration of the anesthetic agent (b) in the whole gas mixture supplied to the inhaler.

When only one vaporizer is to be used the relationship between these variables could be given in simpler equations:

$$A = \frac{F_{k_1} \times P_1}{(760 - P_1)\left\{F_d + F_{k_1}\left(1 + \frac{P_1}{760 - P_1}\right)\right\}} \times 100\% \qquad (5)$$

$$B = \frac{F_{k_2} \times P_2}{(760 - P_2)\left\{F_d + F_{k_2}\left(1 + \frac{P_2}{760 - P_2}\right)\right\}} \times 100\% \qquad (6)$$

As P_1 and P_2 are constant with each agent at the particular temperature, A and B are dependent upon F_d, F_{k_1} and F_{k_2} in the formulas (1) and (2). On the contrary, if the desired concentrations of each drug (A, B) mixing ratio (A/B), and diluent flow (F_d) are given F_{k_1} and F_{k_2} are obtained from the equations (1), (2), (3) and (4).

As a direct calculation of desired values from these equations are somewhat complex and time-consuming, it seems to be necessary to provide a simple flow-temperature-concentration nomogram or a calculator for the convenience of practical use of this machine.

Table I.

Temperature °C \ V/V Ratio	1% Fluothane-Ether Mixture										
	0/100	10/90	20/80	30/70	40/60	50/50	60/40	70/30	80/20	90/10	100/0
15	0 58	15 51	30 45	45 40	60 35	74 29	89 23	104 17	118 11	133 6	150 0
16	0 54	14 48	28 43	42 38	56 32	71 27	85 22	99 16	113 11	128 5	142 0
17	0 49	13 45	26 40	40 35	53 30	66 25	80 20	93 15	107 10	120 5	134 0
18	0 45	12 40	25 36	37 32	50 27	63 23	75 18	88 14	101 9	113 5	126 0
19	0 41	12 37	23 33	35 29	47 25	59 21	71 17	82 13	94 8	106 4	118 0
20	0 38	11 34	22 30	34 27	45 23	55 19	66 15	77 11	89 8	100 4	111 0
21	0 34	10 31	21 28	31 24	41 21	52 17	62 14	73 10	83 7	94 4	104 0
22	0 31	10 28	19 25	29 22	39 19	49 16	58 13	68 9	78 6	88 3	98 0
23	0 28	9 25	18 23	27 20	36 17	46 14	55 12	64 9	73 6	83 3	92 0
24	0 25	9 22	17 20	26 17	34 15	43 13	51 10	60 8	69 5	77 3	86 0
25	0 23	8 20	16 18	24 15	32 13	40 11	48 9	56 7	64 4	72 2	80 0
26	0 19	8 17	15 16	23 14	30 12	37 10	45 8	53 6	60 4	68 2	75 0
27	0 17	7 15	14 13	21 12	28 10	35 8	42 7	49 5	56 3	62 2	70 0
28	0 14	7 13	13 12	19 10	26 9	32 7	39 6	46 4	52 3	60 2	65 0
29	0 12	6 11	12 9	18 8	24 7	30 6	36 5	42 4	48 2	54 1	61 0
30	0 9	6 9	11 8	17 7	22 6	28 5	34 4	39 3	45 2	51 1	56 0

Table I is a flow-temperature-concentration nomogram prepared for fluothane-ether anesthesia. Since it is impossible to summarize many factors such as E_d,

F_{k_1}, F_{k_2}, P_1 P_2, A and B, etc. into a single chart, F_{k_1} and F_{k_2} (cc./min.) required to produce 1% fluothane-ether mixture of various mixing ratio at the temperature range of 15–30° C when F_d is set to 5000 cc./min., are shown in Table I.

On the practical application of Table I, a few simple calculations are necessary as the nomogram is prepared for the case in which F_d and the sum of concentration of fluothane and ether are fixed to 5000 cc./min. and 1% v/v respectively.

(1) In a case in which F_d other than 5000 cc./min. is desired, for example m×5000 cc./min., then multiply the F_{k_1} and F_{k_2} by m.

(2) When the concentration of fluothane-ether mixture other than 1% is desired, for example n% FEM, then multiply the F_{k_1} and F_{k_2} by n. Errors produced by this calculation are negligible, provided that F_d is large enough in comparison with F_{k_1} and F_{k_2}.

(3) When temperature of fluothane and ether are different from each other, pick up the F_{k_1} and F_{k_2} at the respective working temperature from Table I. Errors are quite small with this calculation, as the temperature difference remains usually within a few degrees centigrade. (refer to the formulas (1) and (2)).

The descriptions of concentration and mixing ratio are somehow complex and need to be explained.

The following expressions are commonly used in our laboratory. For example :

Ex. 1. 2% FEM 50/50 Ex. 2. 3% CEM 33/67

Ex. 1 represents a gaseous mixture containing 1% fluothane and 1% ether in volume percent and the total concentration of the anesthetic vapors is 2%.

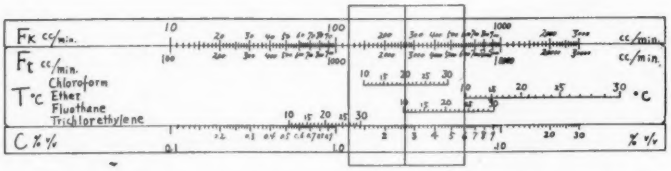

Fig. 2. Flow-Temperature-Concentration calculator for copper kettle vaporizer (Example of calculation 1.)

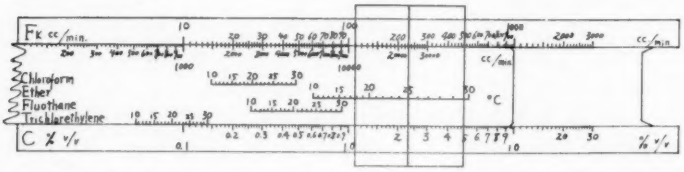

Fig. 3. Flow-Temperature-Concentration calculator for copper kettle vaporizer (Example of calculation 2.).

Ex. 2 represents a gaseous mixture containing 1% chloroform and 2% ether and the total concentration of the anesthetic vapors is 3%. The mixing ratio of chloroform and ether in the latter is 33/67.

More recently, a simple and accurate calculator for copper kettle vaporizers was developed in our laboratory (Patent pending 36–16813).

The basic scales are calibrated for kettle flow (F_k cc./min.) and anesthetic vapor concentration ($C\%$ v/v). The sliding scale is calibrated for total gas flow (F_t cc./min.) and temperature (°C) of various anesthetic agents (chloroform, ether, fluothane and trichlorethylene) as seen in Figs. 2 and 3. In a standard position of the calculator, F_t/F_k is calibrated in a proportion of 10/1.

Examples of calculation are presented.

Ex. 1

Fig. 2 illustrates how to calculate the vapor concentration when the temperature of the anesthetic, total gas flow and oxygen inflow to the copper kettle are given.

If F_k is set to 500 cc./min., F_t to 5000 cc./min. and the temperature of chloroform is 20°C, the vapor concentration of chloroform supplied to the inhaler is about 2.6%(v/v).

This corresponds very well to the theoretically calculated value, 2.62%. It is not necessary to shift the sliding scale in this example of calculation.

Ex. 2

Fig. 3 illustrates an example in which 2.3% (v/v) of ether vapor is desired to be delivered from the anesthetic machine to the inhaler. Temperature of ether is supposed to be 25°C.

Firstly move the cursor and correspond the cursor line to 2.3% on the C scale.

Secondly, shift the sliding scale towards left and correspond the line of 25°C of ether to the cursor line.

Then, $F_k/F_t=1/100$ is obtained on the F_k and F_t scale. When total flow of 51. /min. is desired, oxygen inflow to the vaporizer should be 50 cc./min. in this case.

On the combined use of two volatile anesthetic agents, calculation is made in the same way with respective anesthetic agents separately as described in Exs. 1 and 2.

Instead of "diluent flow." total gas flow was used in this scale in order to make the calculator as compact as possible.

The relationship between total gas flow (F_t) and diluent flow (F_d) is well described in the following formula.

(1) A use of single copper kettle vaporizer

$$F_d = F_t\left(1 - \frac{A}{100}\right) - F_{k_1} \tag{7}$$

(2) Combined use of two copper kettle vaporizers

$$F_d = F_t\left(1 - \frac{A+B}{100}\right) - (F_{k_1} + F_{k_2}) \tag{8}$$

In these formulas marks are used as follows:

F_d(cc./min.): Diluent or bypass flow.

F_t(cc./min.): Total gas flow from the anesthetic machine to the inhaler.

F_{k_1}(cc./min.): Oxygen inflow to the copper kettle vaporizer 1.

F_{k_2}(cc./min.): Oxygen inflow to the copper kettle vaporizer 2.

A(% v/v): Vapor concentration of the anesthetic contained in the vaporizer 1 in the whole gas mixture supplied to the inhaler.

B (%(v/v): Vapor concentration of the anesthetic contained in the vaporizer 2 in the whole gas mixture supplied to the inhaler.

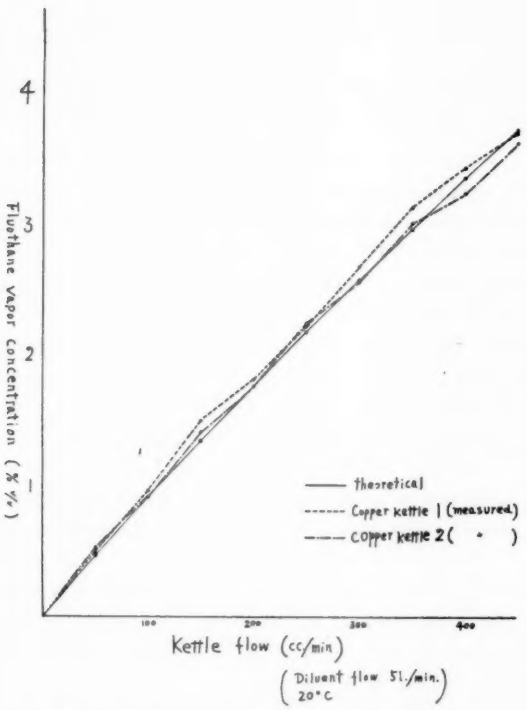

Fig. 4. Performance of copper kettle vaporizers.

Also, when F_{k_1} and a (cc./min. : volume of an anesthetic vapor taken up by the oxygen flow through the vaporizer 1——$F_t \times A/100$ in the formula (7)) are small enough as compared with F_t, F_t could be dealt as F_d without making any significant discrepancies in calculation.

We are using this calculator with great satisfaction in clinical anesthesia and laboratory investigations.

3. Performance characteristics (measured)

Actual vaporization performance of our first machine was described in Figs. 4 and 5. Performance of individual vaporizer in a single use and that of two vaporizers in combined use were tested with dry gas meter and further confirmed with an ultraviolet absorption technique previously described by Kalow[3] and MacKay and Kalow[4] and modified by us.

Fig. 4 illustrates the vernier increments of fluothane vapor concentration in correlation with an increase in oxygen inflow to each copper kettle vaporizer. Bypass flow and temperature of the anesthetic were set to 5l./min. and 20° C

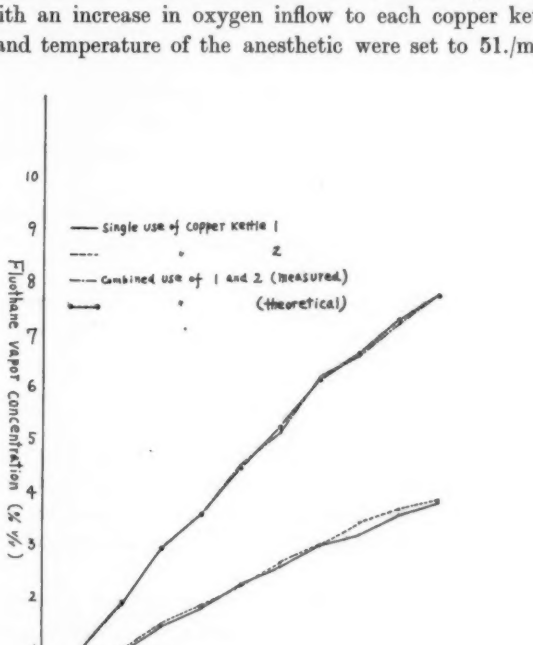

Fig. 5. Combined use of two copper kettle vaporizers

respectively in these measurements.

Actual vaporization of fluothane from two copper kettle vaporizers in combined use was shown in Fig. 5.

Both with a use of single vaporizer and a combined use of two vaporizers, the new double copper kettle machine we tested has a good vaporization performance which is in very close correspondence with that of theoretically calculated.

Both fall in temperature and difference in temperatures of two anesthetic agents in the vaporizers have been almost negligible with our machine when vapor concentration of fluothane-ether mixture or chloroform-ether mixture remained within 2% v/v and the time of administration did not exceed two hours.

However, if higher anesthetic concentrations were used for a long period of time, a fall in liquid temperature and temperature difference between two anesthetic agents might become considerably larger.

Since the latent heat of vaporization of various volatile anesthetic agents are, ether : 87.0 Cal./gm., fluothane : 35.2 Cal./gm., chloroform : 64.0 Cal./gm. at 20°C and the specific volume of vapors of these agents at 20°C are, ether : 324.3cc./gm., fluothane : 121.6 cc/gm., chloroform : 201.6.cc./gm., heat for vaporization required for production of 1l. of anesthetic vapor are, ether : 267.9 Cal., fluothane : 369.3 Cal. and chloroform : 317.4 Cal.

If the equal vapor concentration is used, a degree of fall in temperature due to vaporization must be in the following order :

Fluothane>Chloroform>Ether

In our first trial model, one vaporizer was made a little larger than the other in order to minimize the discrepancy in temperature of two volatile anesthetic agents during practical application.

SUMMARY AND CONCLUSION

A method and an apparatus for simultaneous administration of two or more volatile anesthetic agents under precise and expedient control of vapor concentration and mixing ratio were described in details and problems on performance characteristics of the newly designed double copper kettle machine were discussed in the present paper.

An anesthetic apparatus equipped with two copper kettle vaporizers was introduced by the Foregger Company a few years ago[5]. This is, however, designed for a single use of different volatile anesthetic agents and it is impossible to deliver vapors of two volatile anesthetic agents simultaneously under precise and expedient control of concentration and mixing ratio. This is essentially a different apparatus from the new double copper kettle machine.

It is now possible to do a "precision anesthesia" using two volatile anesthetic agents with this apparatus.

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Studies on the Antibiotic-susceptibility of the Isolates from Staphylococcal Infections

By

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(Received for publication, June 22, 1961)

A large number of reports appearing in scientific literatures clearly indicate the importance of hospital staphylococcal infection. Although some studies carried out in the laboratory indicate the increasing tendency of antibiotic-resistant isolates particularly against penicillin and tetracyclin, no further works have been conducted to see the different susceptibility of the isolates obtained from different sources.

This work was conducted in order to obtain a perspective view on the possible occurence of hospital staphylococcal infection in our clinic. The period of examination was from December, 1957 to March, 1961. The degree of resistance against five antibiotics was compared among the isolates obtained, first from hospitalized patients receiving operation, second from out-patients of this clinic, and third from out-patients of other hospitals locating in the suburbs of Sendai City.

As the results, differences in the degree of susceptibility, particularly against streptomycin, tetracycline, and chloramphenicol, have been revealed among the isolates obtained from the three sources. Regardless of the source of isolation, almost all of the strains were found to be resistant to penicillin. Whereas all of the strains so far tested were still susceptible to erythromycin.

EXPERIMENTAL METHODS AND PLANS

1) Susceptibility test

All of the staphylococci examined here were isolated from the inflammatory region of surgical suppurative diseases on either blood agar or nutrient agar plate. All of the isolates were determined to be staphylococcus aureus. They were then stored on nutrient agar slants and tested for their susceptibility to penicillin (PC), streptomycin (SM), chloramphenicol (CP), tetracycline (TC) and erythromycin (EM) by means of agar dilution technique. Concentrations of antibiotics contained in the nutrient media of pH 7.4 were 100, 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8,

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0.4, 0.2 and 0.1u/ml with PC and mcg/ml with the remaining four antibiotics. In all assays FDA 209 P strain of staphylococcus was involved as the reference standard to verify the result.

2) Criterion of degree of susceptibility

The incidence of the "sensitive", "moderately resistant" and "resistant" was calculated according to the criterion proposed by Eisenberg *et al.*[1] In principle, it was determined on the basis of possible maximum blood level of the antibiotics after administration of the clinical doses. They are as follows;

	"Sensitive"	"Moderately resistant"	"Resistant"
PC (u/ml)	≤ 0.8	1.6~50	≥ 100
SM (mcg/ml)	≤ 12.5	25~50	≥ 100
CP (mcg/ml)	≤ 6.3	12.5~50	≥ 100
TC (mcg/ml)	≤ 3.1	6.3~50	≥ 100
EM (mcg/ml)	≤ 1.6	3.1~50	≥ 100

3) Experimental plans

This study was conducted firstly to see the different susceptibility of 500 isolates obtained from three different environments. These strains were obtained from December, 1957 to March, 1961. 133 strains of Group 1 were obtained from hospitalized patients receiving operations of our university clinic. 188 strains of Group 2 were obtained from out-patients who visited our clinic. 179 strains of Group 3 were obtained from out-patients who visited hospitals locating in the suburbs of Sendai City.

Secondly, the result obtained with the Group 1 strains were rearranged to see the yearly changes of resistant proportions among isolates.

Thirdly, with some strains which were isolated from the patients receiving large doses of antibiotics, the possible increase in the degree of resistance has been examined.

EXPERIMENT

Susceptibility studies of 500 staphylococcal isolates

All of the five hundred isolates were tested for their susceptibility to respective antibiotics as described in the section of experimental method. Protocol of the result was shown in Table I. On the basis of this result, the incidence of the "sensitive", "moderately resistant" and "resistant" was calculated (Fig. 1). One can find from the figure that almost all of the isolates are sensitive to EM, whereas the contrary is the case with PC.

Against PC, only 9.8 per cent of the isolates were sensitive, whereas the highest incidence of resistance such as 57.2 per cent was obtained. With SM, the resistant strains were found in 37.2 per cent. However, the susceptibility

TABLE I. In Vitro Susceptibility of 500 Strains of Staphylococci

Antibiotics		M. I. C. (u. or mcg/cc)											
		>100	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0.1≧
PC	No. of strains	203	83	53	31	40	15	15	11	13	9	5	22
	(%)	(40.6)	(16.6)	(10.6)	(6.2)	(8)	(3)	(3)	(2.2)	(2.6)	(1.8)	(1)	(4.4)
SM	No. of strains	156	30	22	32	50	79	78	40	8	3	1	1
	(%)	(31.2)	(6)	(4.4)	(6.4)	(10)	(15.8)	(15.6)	(8)	(1.6)	(0.6)	(0.2)	(0.2)
CP	No. of strains	4	30	38	37	116	167	69	23	11	3	2	0
	(%)	(0.8)	(6)	(7.6)	(7.4)	(23.2)	(33.4)	(13.8)	(4.6)	(2.2)	(0.6)	(0.4)	(0)
TC	No. of strains	74	20	17	13	34	106	119	60	37	12	4	4
	(%)	(14.8)	(4)	(3.4)	(2.6)	(6.8)	(21.2)	(23.8)	(12)	(7.4)	(2.4)	(0.8)	(0.8)
EM	No. of strains	0	0	1	2	4	2	2	11	48	140	143	147
	(%)	(0)	(0)	(0.2)	(0.4)	(0.8)	(0.4)	(0.4)	(2.2)	(9.6)	(28)	(28.6)	(29.4)

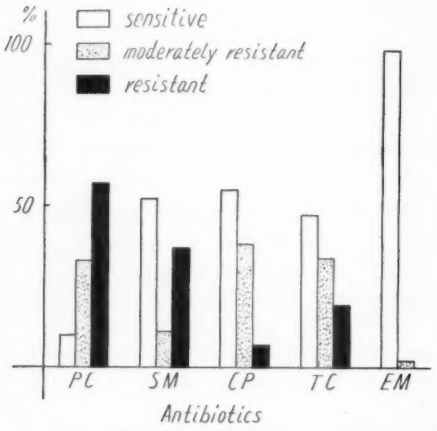

Fig. 1. Antibiotic spectrum of 500 strains of staphylococci.

of the remaining organisms revealed the peak in the concentration of 3.1 to 6.3 mcg/ml (see Fig. 2).

Fig. 2 is the distribution curve of minimum inhibitory concentration determined with each isolate against each antibiotic. In Fig. 2, the same kind of distribution was revealed against SM, TC and CP. However, when the curve obtained against CP was examined in detail, there were no highly resistant strains. This is again evident from Fig. 1. Only 6.8 per cent of the tested was defined to be resistant. The curve of susceptibility against TC resembled that of SM,

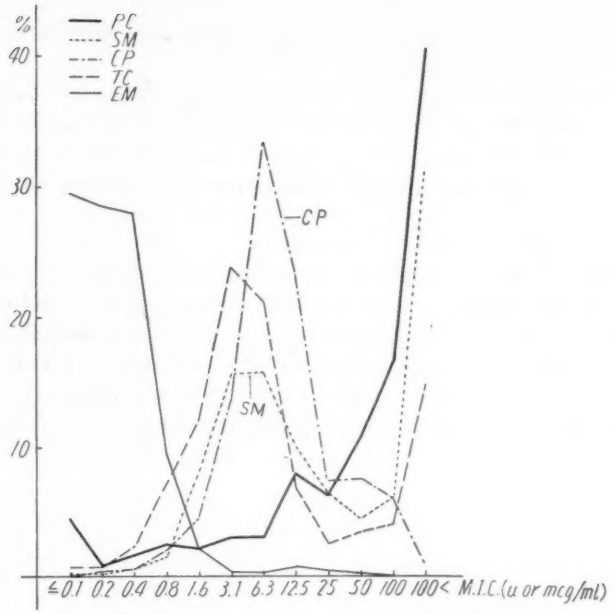

Fig. 2. Susceptibility of 500 isolates to five antibiotics.

and the peak was at 3.1 to 6.3 mcg/ml. Isolates resistant to TC were found in 18.8 per cent (Fig. 1). This incidence was apparently less than that of SM but higher than that of CP. The strains sensitive to these antibiotics were at almost similar levels of, in the order of frequency, 55 per cent to CP, 52 per cent to SM, and 47.2 per cent to TC respectively.

The frequency to meet multiple resistant strains is said to be gradually increasing particularly with those obtained from inflamation areas. To test this possibility, incidence of the strains which were resistant to two or more antibiotics was analysed. As a result, the multiple resistant strains numbered 165 of the 500 (33 per cent).

The strains resistant to two antibiotics reached 97 of the 165, the majority being resistant to both PC and SM (73 per cent) followed by the strains resistant to SM and TC (14 per cent), strains resistant to PC and TC (10 per cent), and a few strains resistant to other combinations of two antibiotics, i.e. CP-TC (2 per cent) and PC-CP (1 per cent).

Of 44 strains resistant to three antibiotics, 40 (90 per cent) showed resistance to the combination of PC, SM and TC, and the isolates resistant to the combination of PC-SM-CP and SM-CP-TC were found only 1 (3 per cent) and 3 (7 per cent)

respectively.

All of the 24 strains resistant to four antibiotics were resistant to the combination of PC, SM, CP and TC.

When the source of the multiple resistant isolates is taken into consideration, it should be noted that, of the 24 strains resistant to four antibiotics, 20 strains were isolated from the hospitalized patients of our clinic (Group 1), suggesting high frequency to meet the multiple resistant strains in hospital staphylococcal infection.

Environmental effect on antibiotic susceptibility

To compare the susceptibility of staphylococci isolated from different environments, 500 strains were divided into three groups as mentioned in the section of experimental plans. Group 1 was selected to see the high incidence of resistant strains in hospital, especially after receiving operation. Groups 2 and 3 were isolated from out-patients of the city and suburbs, respectively. The distribution curve of M.I.C. of the isolates from these three groups for each

Fig. 3. Susceptibility of three groups to PC.

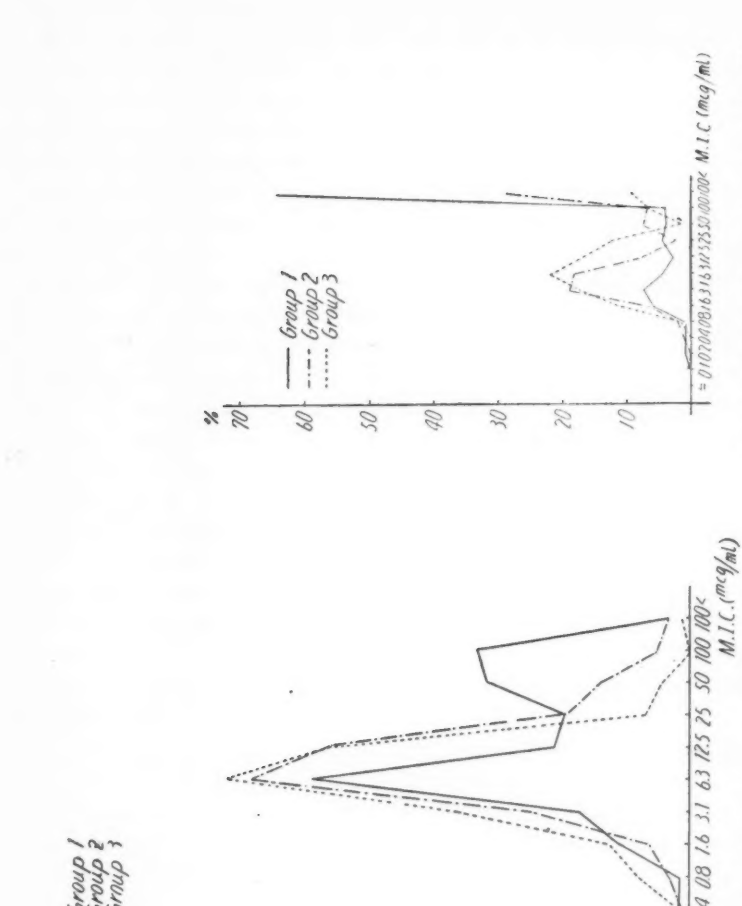

Fig. 5. Susceptibility of three groups to CP.

Fig. 4. Susceptibility of three groups to SM.

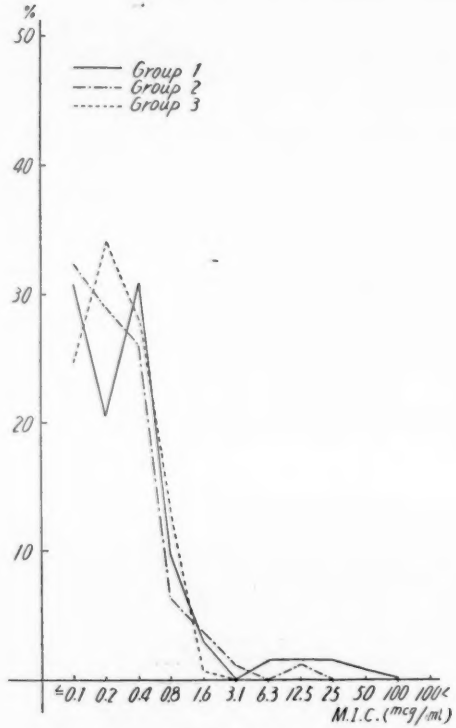

Fig. 7. Susceptibility of three groups to EM.

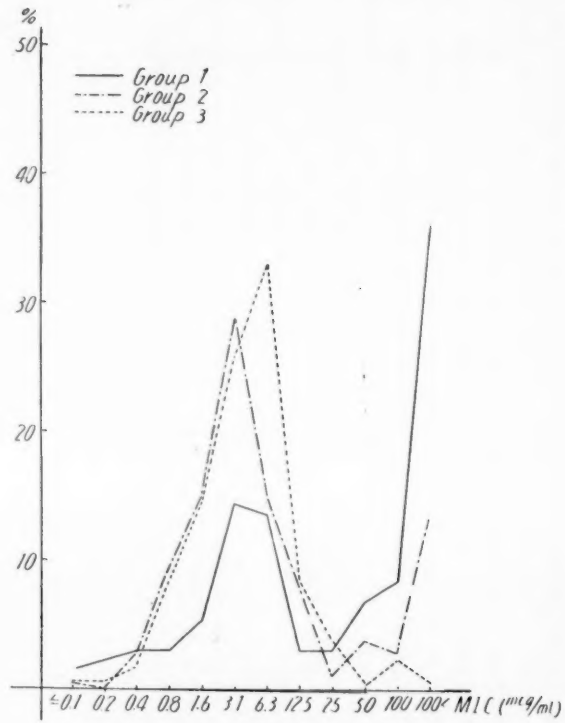

Fig.6. Susceptibility of three groups to TC.

five antibiotics was illustrated in Figs. 3–7.

The result with PC was shown in Fig. 3. There were no significant differences among the three groups, suggesting high incidence of resistant strains not only in hospital but also among out-patients.

With SM, as shown in Fig. 4, a symmetrical curve with a peak at the concentration of 6.3 mcg/ml was seen in Group 3, and the resistant strains were 16.4 per cent, whereas in Group 1, no peak was seen in the vicinity of 6.3 mcg/ml and the incidence of the resistant strains was as high as 68.5 per cent. Group 2 gave a curve lying between that of Group 1 and Group 3. The resistant strains were found in 35.1 per cent.

With CP, as shown in Fig. 5, Groups 2 and 3 showed almost similar curves which are steep and symmetrical with a peak at 6.3 mcg/ml, and the incidence of the resistant isolates was very low. On the other hand, in Group 1, two peaks at 6.3 mcg/ml and 50 to 100 mcg/ml were found.

With TC, as illustrated in Fig. 6, Group 3 showed a steep and symmetrical curve with a peak at 3.1 to 6.3 mcg/ml, and only few strains (0.6 per cent) required more than 100 mcg/ml for inhibition. In Group 2, although the resistant strains were seen in 16.0 per cent, a peak at 3.1 mcg/ml was clearly recognized. In Group 1, the corresponding peak was far lower than in other groups, while the incidence of the resistant strains was 44.4 per cent.

With EM, as shown in Fig. 7, the curves of three groups were quite similar to each other and the majority of the strains were inhibited by a low concentration.

Increasing tendency of resistant strains against PC, TC and CP in hospital staphylococcal infections

To investigate the yearly changes of antibiotic susceptibility, 100 strains were selected out of 133, which were isolated from hospitalized patients. They were divided in two, one being the isolates obtained between May, 1958 and January, 1959 and another was obtained from July, 1959 to March, 1961. The results obtained were shown in Table II.

As for PC, the numbers of the sensitive and moderately resistant strains decreased, while the incidence of the resistant strains increased from 50 per cent to 70 per cent.

With SM, no significant changes were observed between these two periods.

As for CP, although no changes were seen in the incidence of resistant strains, the sensitive strains decreased from 60 per cent to 40 per cent and the moderately resistant strains increased significantly from 20 per cent to 44 per cent.

With TC, although there were no significant changes in the incidence of resistant strains between the two periods, a tendency of a decrease in the sensitive strains and an increase in the moderately resistant strains was suggested.

TABLE II. Antibiotic Spectrum of Staphylococci Isolated from Inpatients at the Earlier Period (50 Strains) and the Later Period (50 Strains)

Antibiotics		No. of sensitive strains (%)	No. of moderately resistant strains (%)	No. of resistant strains (%)
PC	Former period	9 (18)	16 (32)	25 (50)
	Later period	3 (6)	12 (24)	35 (70)
SM	Former period	11 (22)	5 (10)	34 (68)
	Later Period	13 (26)	5 (10)	32 (64)
CP	Former period	30 (60)	10 (20)	10 (20)
	Later period	20 (40)	22 (44)	8 (16)
TC	Former period	19 (38)	11 (22)	20 (40)
	Later period	15 (30)	16 (32)	19 (38)
EM	Former period	48 (96)	2 (4)	0 (0)
	Later period	46 (92)	4 (8)	0 (0)

With EM, no significant changes were observed in the incidence of the sensitive strains between two periods.

Antibiotic resistance of the isolates obtained after the administration of large doses of antibiotics

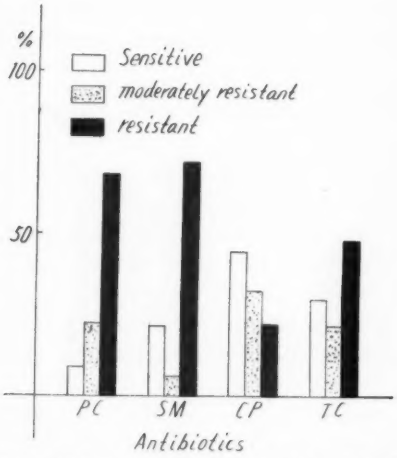

Fig. 8. Antibiotic spectrum of staphylococci isolated from patients administered with a large dose of antibiotics.

To study the relation between the amount of antibiotics already administered and the susceptibility of staphylococci, an assay was done on the strains isolated

from the patients who had received more than 3,00,000 units of PC or more than 5 gm. of SM, CP or TC for treatment. The results are shown in Fig. 8. The assay with EM was omitted because of the high susceptibility obtained with the tested strains.

When Fig. 8 is compared with Fig. 1, which expresses the susceptibility of total number of 500 strains, general impression is the increased incidence of resistant strains and the decreased incidence of sensitive strains after administration of a large amount of antibiotics. This was particularly prominent with the strains exposed to a large amount of SM or TC ; the strains resistant to SM were 71.8 per cent and to TC 48 per cent, and the sensitive strains were seen in only 21.8 per cent to SM and 30 per cent to TC.

DISCUSSION

From 1951 to 1955, Spink[2][3] studied the yearly changes of antibiotic susceptibility of staphylococci at the Mineapolis General Hospital. According to his investigation, susceptibility of staphylococci to PC showed almost no yearly changes and the incidence of the resistant strains not inhibited by 50 u/ml of PC was 70.8 per cent in 1955. Concerning SM, the incidence of the strains resistant to 50 mcg/ml increased from 48.0 per cent in 1951 to 69.38 per cent in 1955. The incidence of TC resistant strains increased by the end of 1953, but showing no significant changes since then, and 49.76 per cent of the strains were resistant to TC in 1955. On the contrary, he found only a few CP resistant strains (1.91 per cent in 1955). With regard to this matter, he explained that this finding was no doubt related to the fact that CP had not been widely used in the hospital because of apprehension concerning its toxic activity on the bone marrow. He also stated that no EM resistant strains were seen in 1952, while there were found 22.49 per cent in 1955.

Also in Japan, the investigation throughout the country by the Staphylococcal Susceptibility Committee of National Hospitals[4] from August, 1957 to July, 1959 showed a similar tendency, and the incidence of antibiotic resistant staphylococci was increasing year after year. The incidence of CP resistant strains did not show increasing tendency as in the U.S.A. However, EM resistant strains were scarcely seen unlike in the U.S.A.

Of the 500 strains examined in this experiment, none was resistant to EM and only 2.2 per cent were moderately resistant to EM. It is conceivable that the lower incidence of EM resistant strains in Japan is due to the fact that this antibiotic has been used only to a limitted degree in Japan and there have been less chances for staphylococci to be exposed to EM. Possible increase of EM resistant strains may be suggested, when the increasing use of EM in Japan is taken into consideration.

On the other hand, the incidence of CP resistant strains was very low in spite

of that this antibiotic had been generally used in Japan. This may be attributed to the fact that staphylococci hardly become resistant to CP in nature. With this regard, Finland[5] and Balch[6] are stating the same opinion, and in Wright's[7] observation, staphylococci becoming gradually resistant to PC, SM and TC did not become resistant to CP.

The author's results revealed that 34 strains out of 500 (6.8 per cent) were resistant to CP, the incidence being higher than that of Spink's investigation in 1955. The number of CP resistant strains is not so great as to cause troublesome clinical problems, but it is to be noted that, as shown in Fig. 5, the strains isolated from hospitalized patients of our clinic showed a tendency of acquiring resistance to CP.

Of the 500 strains in this experiment, strains resistant to 1.6 u/ml of PC were seen in as many as 90.2 per cent. However, considering the fact that the strains isolated from three different environments showed almost similar susceptibility as seen in Fig. 3, it seems likely that the tendency of increasing staphylococcal resistance to PC is almost terminated.

Concerning SM and TC, the resistant strains have gradually increased in Japan in these several years, but the incidence is not so remarkable as in the U.S.A. In the author's investigation, the strains resistant SM and TC were seen in 37.2 per cent and 18.8 per cent, respectively, the incidence being lower than that of Spink's investigation in 1955, but an increased incidence of the resistant strains should be expected in the future.

Recently clinical interest has been directed to the strains which are resistant not only to PC but also to the other antibiotics. Ueda[8] investigated the yearly changes of the incidence of multiple resistant staphylococci from 1957 to 1960 and recognized a yearly increase of the multiple resistant strains.

Of the 500 strains under discussion, 165 strains were multiple resistant to the combination of 50 u/ml or mcg/ml of antiboitics. Most of the combinations included either PC or SM.

It is already recognized that susceptibility of staphylococci shows geographical and environmental differences.

In the author's experiment of 500 strains, there was no remarkable difference of susceptibility to PC among three different environmental groups. This is perhaps due to the common use of PC, and for the opposite reason that EM is not popular yet, no environmental difference in susceptibility to EM was seen.

With CP, the majority of the strains of Groups 2 and 3 were inhibited by 12.5 mcg/ml or less, but a considerable number of the strains of Group 1 required 50 to 100 mcg/ml for inhibition. This suggests that CP resistant strains are also gradually increasing.

With SM and TC, a large difference was seen between the strains of Groups 3 and 1, the incidence of resistant strains being remarkably high in the latter.

These findings with CP, SM and TC are perhaps due to the larger amount of antibiotics used in the university hospital, and the smaller difference with CP is due to the fact that staphylococci hardly becomes resistant to CP.

When the susceptibility of the early isolated 50 strains and the lately, isolated 50 strains was compared, no increase in the incidence of CP or TC resistant strains was seen, whereas a decrease of CP or TC sensitive strains and an increase of CP or TC moderately resistant strains were recognized.

Many reports have been appeared concerning the changing susceptibility of staphylococci isolated after administration of a large amount of antibiotics. Some of them are affirmative to the fact, whereas others are not.

In the author's investigation, susceptibility of the strains isolated after use of a large amount of antibiotics showed a decreased incidence in the sensitive strains and an increased incidence in the resistant strains. This difference was most remarkable with SM, suggesting that staphylococci became most easily resistant to SM *in vivo*.

SUMMARY

The antibiotic susceptibility of 500 strains of staphylococcus aureus isolated from the patients with surgical suppurative diseases from December, 1957 to March, 1961 was studied.

The results obtained will be summarized as follows:

1) Only a few strains were inhibited by a low concentration of penicillin. On the contrary, the majority of the strains were inhibited by a low concentration of erythromycin. Distribution curves of susceptibility to streptomycin, chloramphenicol and tetracycline lay between those of penicillin and erythromycin.

The incidence of the resistant strains to five antibiotics was, in the order of frequency, 58.2 per cent to penicillin, 37.2 per cent to streptomycin, 18.8 per cent to tetracycline, 6.8 per cent to chloramphenicol and none to erythromycin.

Multiple resistant strains numbered 165, the majority of them being resistant to both penicillin and streptomycin.

2) Environmental and geographical influence on staphylococcal susceptibility was studied with three different groups of strains.

With streptomycin and tetracyclin, there was a distinct difference between the strains isolated from hospitalized patients of our clinic and the strains isolated from the out-patients of suburban hospitals, the incidence of resistant strains being remarkably high in the former.

With chloramphenicol, a considerable number of the strains isolated from hospitalized patients of our clinic required a high concentration for inhibition, although not so remarkable as with streptomycin and tetracycline.

With penicillin and erythromycin, there was no remarkable difference in

the susceptibility of staphylococci among the three groups.

3) Of the 133 strains isolated from the hospitalized patients of our clinic, the susceptibility of 50 strains isolated between May, 1958 and January, 1959 and of 50 strains isolated between July, 1959 and March, 1961 were compared.

The penicillin resistant strains moderately increased, whereas with streptomycin and erythromycin, no significant changes were seen between the two groups. With chloramphenicol and tetracycline, no increase in the incidence of resistant strains was seen, but a decrease of sensitive strains and an increase of moderately resistant strains were seen in the lately isolated group.

4) As for the changes of the susceptibility of staphylococci after administration of a large amount of antibiotics, a decrease of sensitive strains and an increase of resistant strains were seen with every one of five antibiotics, the tendency being most remarkable with streptomycin.

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The Effect of Androgen on Fine Structure of the Prostate of Castrated Rats

Report I. The Effect by Administration of Small Dosis of Testosterone One Month after Castration

By

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INTRODUCTION

It is well known that administration of androgen causes the increase of weight and recovery of function of the prostatic gland of castrated rats, and this effect is availed of the prostate-weight method for quantitatively assaying androgen. Thus, it is clear that androgen is closely correlated with the prostate and it may be easily inferred that even a microquantity of androgen causes change in the prostatic epithelium of castrated animals. There are comparatively few works on the epithelial cells of prostate as viewed under an electron microscope, and no report is available on the subject concerning administration of androgen after castration. So, the author castrated male rats, and one month thereafter, gave androgen, to examine the subsequent changes in the prostatic epithelial cells, as presented in the following.

MATERIALS AND METHODS

Ten pure Wistar-breed male rats were divided into the following 5 groups: Group I of mature controls of about 120 g body weight: Group II of rats castrated 3 weeks after birth and then fed for about 1 month till they came to weight ca 120 g; rats similarly castrated, fed and injected with 0.1 ml of olive oil daily — 0.7 ml of which contains 1 μg of testosterone (Group III), 10 μg (Group IV) and 100 μg (Group V) — for 7 consecutive days subcutaneously at the back.

These animals were decapitated, those in Groups III, IV and V 24 hours after the last injection. Their prostate was immediately excised, then fixed in 1% osmic acid solution adjusted to pH 7.4 with Palade's veronal-acetate buffer[1] in an ice-box for 3 hours, washed with water several times and dehydrat-

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ed in a series of ethanol solutions. For embedding, a mixture of 80% of n-butylmethacrylate and 20% of methylmethacrylate was applied and polymerized with benzoil peroxide, at 47°C for 10 hours. Thin sections were cut with glass knives, then stained with 10% phosphotungstic acid[2] and examined under an electron microscope.

RESULTS

1. Findings in Group I (Figs. 1 and 2)

Braunsteiner[3], Harkin[4], Soda[5] and Yamaguchi[6] have reported on the electron microscopic findings of the prostate of normal rats, and the findings of the present author were nearly the same. The epithelial cells were found tall and columnar and containing basal nuclei, provided with microvilli at the luminar border. The endoplasmic reticulum was very well developed and arranged nearly lamellarly, particulary notable in the supranuclear portion, and having Palade's particles. The mitochondria were spherical, elliptical or small rod-like formations, frequently provided with cristae. The Golgi's complex was found not so well-defined as described by the past authors. Large-sized dense granules, as Brandes found in the mouse prostate[7], perhaps corresponding to Yamaguchi's lipoid bodies, were also present. The nuclei were spherical or elliptical, minute granules diffusely filled the nucleoplasm and often nucleoli were found in them. The stroma bounded by the basement membrane from the epithelial cells, containing blood vessels, collagen bundles and connective tissue, was rather small in quantity.

2. Findings in Group II (Figs. 3 and 4)

The findings were little different from those reported by Yamaguchi. The cells were generally atrophied, lowered in height, and the size of nucleus being not much reduced, the cytoplasm was as much obviously diminished. The electron density was lowered so that the cells looked more transparent. The microvilli were far smaller and irregularly arranged. Nearly no endoplasmic reticulum could be observed. The mitochondria were extremely small-sized and had their internal structure worse defined, but in its number and distribution no great difference from the normal was observed. Microvesicles were often present, particularly frequent near the luminar border of the cells. The Golgi's complex was somewhat better defined than in Group I. The nucleoplasm showed the appearance of granules coagulated at its margin.

3. Findings in Group III (Figs. 5 and 6)

The size of cells was nearly equal with that in Group II. The electron density of the cytoplasm was slightly enhanced, the atrophy seemingly being a little alleviated. The microvilli were very small and deranged, the mitochondria

were a little enlarged, were frequently round and had ill-definable internal structure. Vesicle was also frequent. The cell membranes of adjoining cells showed obvious interdigitation. The granules in the nucleoplasm were somewhat denser and the nuclear membrane was notably uneven.

4. Findings in Group IV (Figs. 7 and 8)

Not much different from the findings in Group III but spherical vesicles were visible near the luminar border. Dense granules, apparently lipoid bodies, were also often present. The mitochondria were mostly of the shape of small rods, and some of them contained ill-defined cristae.

5. Findings in Group V (Figs. 9 and 10)

The findings here were perceptibly different from those in Groups III and IV. The epithelial cells was found somewhat taller, and the cytoplasm was similar to that of normal cells. The microvilli are still small and deranged, but the endoplasmic reticulum was revived in the supranuclear portion and sometimes show small lamellar arrangement. Palade's particles were obvious. The mitochondria were generally small-sized and showed nearly the normal findings, with clear-cut cristae. The microvesicles were numerous and conspicuous near the luminar border, but the Golgi's complex was not so well-defined. Most of the nuclei were spherical and the granules in the nucleoplasm showed findings ranging from conglomeration to normal diffusion.

DISCUSSION

Past reports on electron microscopic examination of the prostate are rather few, and we may enumerate those by Braunsteiner *et al.* and Harkin treating the rat prostate, by Brandes *et al.*[7)-9)] treating the coagulating gland and the ventral lobe of mice, and in Japan by Soda and Yamaguchi. Braunsteiner *et al.* studied the findings 8 days after castration and after administration of testosterone, of the large dosis of 25 mg, Harkin pursued the changes after castration day by day, but does not touch upon the findings after administration of testosterone. Soda made experiments with testosterone but used uncastrated animals, while Yamaguchi followed up the change after castration minutely and spoke on the anti-androgenic therapy, but not on experiments with androgenic hormones. Thus, as far as we are aware, there is no report available on an experiment with small dosis of androgen on previously castrated animals.

The present author administered testosterone 1 month after castration, when the changes due to the castration are presumably developed to the maximum. There may be some problem in the mode of administration. The present author used it in olive oil solution, as in the prostate-weight method of assay, and 1, 10 and 100 μg of tetosterone was injected through 7 days and the prostate was excized on the 8th day.

As detailed above, the findings in animals 1 month after castration were, as reported by Harkin and Yamaguchi, those of advanced atrophy. The fundamental bodies were totally degenerated and annihilated, namely the endoplasmic reticulum which forms very beautiful pattern in normal rats was nearly invisible, and the mitochondria were notably reduced in size and the cristae in them were ill-defined. Now, when a small quantity of testosterone was administered, distinct findings of recovery were observed only in the cases given 100 μg of it, those given 1–10 μg barely showing signs of rise of electron density of the cytoplasm, appearance of endoplasmic reticulum in some parts, enlargement of mitochondria and appearance of vesicles, but not in a degree to mark a clear line of demarcation. In short, for recovery from the degenerated state of fine structure of cells one month after castration apparently requires a considerable quantity of testosterone.

SUMMARY

Electron microscopic examination of the prostatic epithelium of castrated rats with or without subsequent administration of testosterone led to the following results.

1. In rats castrated and left untreated for a month, the epithelial cells were evidently atrophied, the electron density of the cytoplasm was lowered, the endoplasmic reticulum was faded out, and the mitochondria were reduced in size.

2. When such rats a month after castration were given per cutem 1–10 μg of testosterone solution in olive oil, no perceptible finding of recovery from the changes was obtained, but after 100 μg of it, the reappearance of endoplasmic reticulum was obvious, the mitochondria were re-enlarged and their internal structure was again definitely observable.

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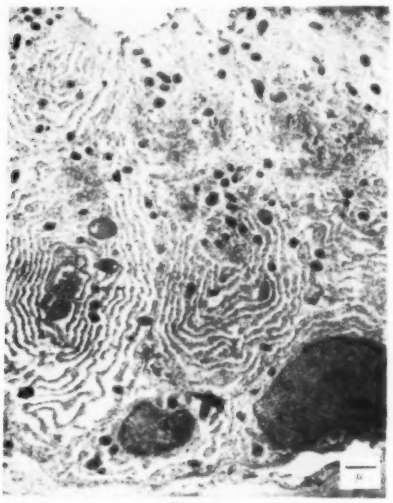

Fig. 1

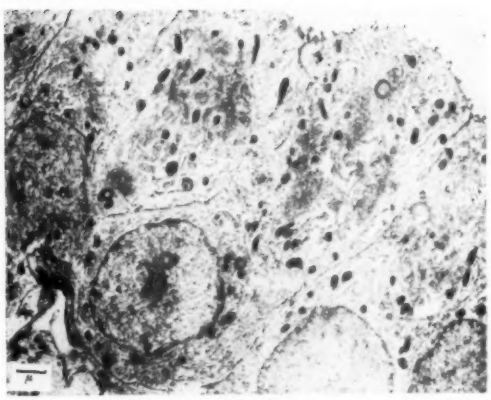

Fig. 2.

Figs. 1 and 2. Normal prostate.

Rough-surfaced endoplasmic reticulum of parallel or vortical arrangement are obvious. The Golgi's complex is not very definite. Lipoid bodies in existence.

Fig. 3.

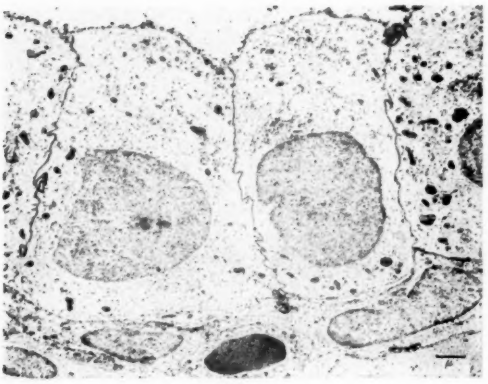

Fig. 4.

Figs. 3 and 4. Prostate 1 month after castration.

The fundamental bodies is scanty and the electron density is low. Endoplasmic reticulum nearly absent. Mitochondria reduced in size. Microvesicles seen especially near the luminar border.

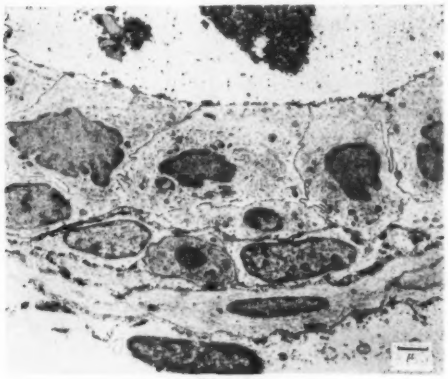

Fig. 5.

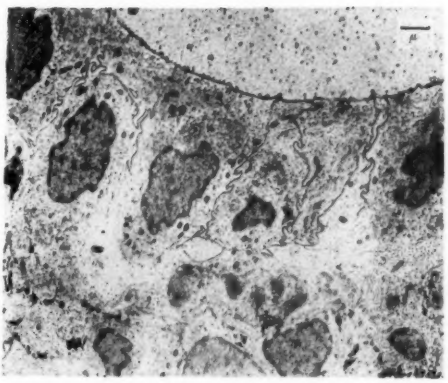

Fig. 6.

Figs. 5 and 6. Prostate administered 1μg of testosterone after 1 month post-castration.

Electron density slightly risen. Mitochondira slightly enlarged. Interdigitation apparent.

Fig. 7.

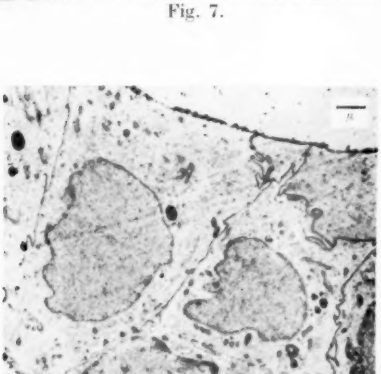

Fig. 8.

Figs. 7 and 8. Ditto after 10μg of testosterone.
Vesicles and lipoid bodies visible. Mitochondria mostly of rod-form.

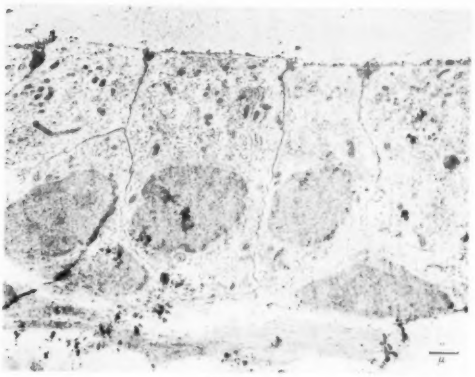

Fig. 9.

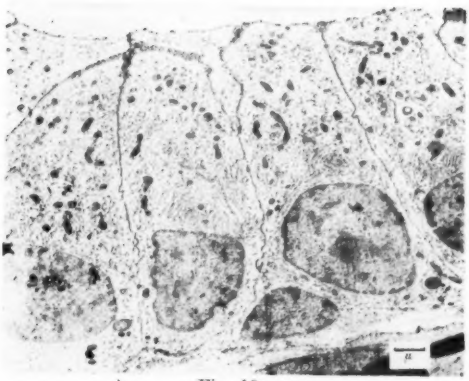

Fig. 10.

Figs. 9 and 10. Ditto after 100μg of testosterone.

Endoplasmic reticulum considerably reappeared. Mitochondria nearly normalized. Numerous microvesicles.

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Salivary Lysozyme : Its Relation to Angular Stomatitis in Ariboflavinosis

Part I.
Salivary Lysozyme Activity in Ariboflavinosis

By

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(Received for publication, June 28, 1961)

It is now well known that angular stomatitis, combined with cheilosis and/or lingual changes, has been not uncommonly found among inhabitants at Hirosaki Area. As to the essense of this affection, I shall not relate it here, but only refer to the paper of Masuda *et al.*[1]

Now I wanted to study whether or not the salivary lysozyme would play any role for the occurrence of the histopathologic changes characteristic of the angular stomatitis associated with ariboflavinosis, and the present report will deal with the salivary lysozyme activity, making the most of the lytic action of lysozyme upon micrococcus lysodeikticus — which action was described by Fleming[2] as the first — in ariboflavinosis of various degree.

METHODS

Quantitative estimation of lysozyme in saliva

The lytic action[3] of lysozyme upon micrococcus lysodeikticus was used for the estimation of lysozyme activity in saliva. Micrococcus lysodeikticus, which had been cultured on the ordinary agar plate for 48 hours at 37°C, was washed twice with distilled water, then with M/15 phosphate buffer of pH 6.9 anew. The microorganisms thus treated were suspended in the phosphate buffer so as to get a suspension of a concentration showing 0.283 of transparency rate at 640 mμ by using Leitz-photometer. The suspension was heated at 100°C for 30 minutes, and after cooling, kept with a piece of thymol added to it in a refrigerator : — This is "the stock suspension of the micrococcus".

To 5.0 cc of "the stock suspension of the micrococcus" was added 0.5 cc of the diluted saliva, which was made by diluting the saliva up to 1 : 5 with the phosphate buffer. The content was mixed well and kept in a water bath at

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38°C for 15 minutes, then kept in an incubator at 38°C for 45 minutes. Immediately after the incubation transparency rate of the suspension was read at 640 mμ by Leitz-photometer. The more active the lysozyme was, the more decreased was the transparency rate of the suspension.

Standard cvrve for the estimation of lysozyme activity in saliva

To five parts of "the stock suspension of the micrococcus" was added 0.5

TABLE I. Transparency Rate of Micrococcus Suspension and Its Relation to Lysozyme Activity.

Turbidity of micrococcus suspension, provisionally expressed in %	Transparency rate	Lysozyme activity (%)
100	0.283	0
90	0.268	10
80	0.244	20
70	0.222	30
60	0.197	40
50	0.171	50
40	0.146	60
30	0.117	70
20	0.079	80
10	0.051	90
0	0.018	100

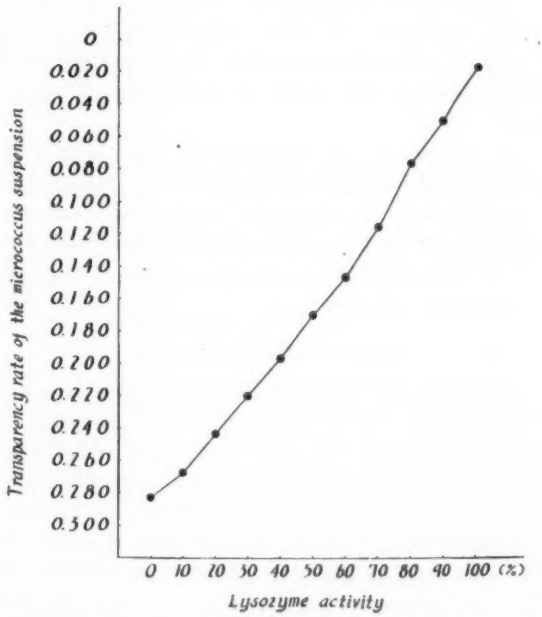

Fig. 1. Standard curve for estimation of lysozyme activity in saliva.

parts of the M/15 phosphate buffer of pH 6.9. The turbidity of the resulting mixture was designed as 100 % arbitrarily, and a series of mixtures with the turbidity of 90, 80, 70, 60, 50, 40, 30, 20, 10 and 0% respectively was prepared by dilution with the phosphate buffer. The transparency rates of each of them, read at 640 mμ by Leitz-photometer, were plotted on a longitude, and % of lysozyme activity (=100% − % of turbidity) were put on an ordinate, thus standard curve for estimation of lysozyme activity in saliva was obtained (cf. Table I and Fig. 1). Suppose lysozyme activity of 50% was found in a given case, it means that lysozyme in the given specimen has an activity to dissolve 50% of the micrococcus contained in the original suspension.

MATERIALS

98 boys, aged from 8 to 12 years, was subjected to this investigation, of whom 32 were healthy and free from ariboflavinosis and the other 66 had some of ariboflavinotic signs. For quantitative evaluation of severity of ariboflavinosis "marks for B_2-deficiency", adovocated by Arakawa[3] from our Laboratory, were used. Only 3 out of many ariboflavinotic signs—angular stomatitis, cheilosis, and lingual changes — were selected as the criterion for the state of B_2-deficiency. Suppose a patient showed angular stomatitis of grade (+), cheilosis (+) (+), and lingual changes (+) (+), the marks for B_2-deficiency of the patient in question were given as 5 (+). This method was useful for clinical estimation of grade of ariboflavinosis, because Arakawa[3] had found a significant reverse correlation between "the marks for B_2-deficiency" and total riboflavin content in blood.

According to "the marks for B_2-deficiency" the boys subjected to this investigation were divided into 7 groups (Groups I–VII) : Group I consisted of the cases without the ariboflavinotic signs, that is, with the marks for B_2-deficiency of O, Group II of those with 1 (+), Group III of those with 2 (+), Group IV of those with 3 (+), Group V of those with 4 (+), Group VI of those with 5 (+), and Group VII of those with 6 (+).

RESULTS AND DISCUSSION

Lysozyme activity of the cases of Groups I–VII was given in Tables II, III and IV (cf. Tables II–IV). As is illustrated in Fig. 2, averages for the salivary lysozyme activity in Groups I–VII were distributed with the connecting line forming a valley-like figure of which the bottom was located in a point corresponding to the average figure of Group IV. Even though no statistically significant differences were found among these averages, noteworthy was such a tendency that a decrease in the salivary lysozyme activity was found in only the cases of slight to moderate ariboflavinosis but not in those of extremely severe ariboflavinosis.

TABLE II. Lysozyme Activity in Saliva of Children without Ariboflavinosis.

Group	No. of case	Age (years)	Lingual change	Angular stomatitis	Cheilosis	Marks for B_2-deficiency	Lysozyme activity in saliva (%)	
I	1	8	–	–	–	0	35	57.8±3.2
	2	8	–	–	–	0	45	
	3	8	–	–	–	0	66	
	4	8	–	–	–	0	63	
	5	8	–	–	–	0	70	
	6	8	–	–	–	0	65	
	7	8	–	–	–	0	65	
	8	8	–	–	–	0	54	
	9	8	–	–	–	0	52	
	10	8	–	–	–	0	60	
	11	10	–	–	–	0	60	
	12	10	–	–	–	0	59	
	13	10	–	–	–	0	52	
	14	10	–	–	–	0	65	
	15	10	–	–	–	0	50	
	16	10	–	–	–	0	46	
	17	10	–	–	–	0	54	
	18	10	–	–	–	0	60	
	19	10	–	–	–	0	51	
	20	12	–	–	–	0	41	
	21	12	–	–	–	0	57	
	22	12	–	–	–	0	64	
	23	12	–	–	–	0	67	
	24	12	–	–	–	0	59	
	25	12	–	–	–	0	45	
	26	12	–	–	–	0	56	
	27	12	–	–	–	0	68	
	28	12	–	–	–	0	55	
	29	12	–	–	–	0	69	
	30	12	–	–	–	0	71	
	31	12	–	–	–	0	65	
	32	12	–	–	–	0	62	

TABLE III. Lysozyme Activity in Saliva of Children with Ariboflavinosis.

Group	No. of case	Age (years)	Lingual change	Angular stomatitis	Cheilosis	Marks for B_2-deficiency	Lysozyme activity in saliva (%)	
II	33	8	–	–	+	1	48	50.0±8.6
	34	10	+	–	–	1	52	
III	35	8	–	+	+	2	61	48.0±5.6
	36	8	–	+	+	2	72	
	37	8	–	+	+	2	35	
	38	8	–	+	+	2	33	
	39	8	–	+	+	2	57	
	40	8	+	–	+	2	69	
	41	8	–	+	+	2	38	
	42	8	+	–	+	2	63	
	43	8	–	+	+	2	38	
	44	10	–	–	±	2	57	
	45	10	–	+	+	2	42	
	46	10	–	–	±	2	30	
	47	10	–	+	+	2	41	
	48	10	–	+	+	2	53	
	49	10	+	–	+	2	54	
	50	11	–	+	+	2	35	
	51	11	+	–	+	2	44	
	52	12	–	+	+	2	37	

	53	12	+	−	+	2	65
	54	12	−	+	+	2	34
	55	12	−	+	+	2	69
	56	12	−	+	+	2	41
	57	12	−	+	+	2	37
IV	58	8	−	++	+	3	46
	59	8	+	+	+	3	52
	60	8	+	+	+	3	62
	61	8	+	+	+	3	44
	62	8	+	+	+	3	51
	63	8	+	+	+	3	44
	64	10	−	+	++	3	39
	65	10	−	+	++	3	48
	66	10	+	+	+	3	69
	67	10	+	+	+	3	38
	68	10	−	++	+	3	22
	69	10	+	−	++	3	21
	70	11	−	+	++	3	38
	71	12	−	++	+	3	22
	72	12	−	+	++	3	65
	73	12	−	+	++	3	73
	74	12	+	+	+	3	46
	75	12	+	+	+	3	52
	76	12	+	+	+	3	37
	77	12	−	+	++	3	35

45.2±14.6

Table IV. Lysozyme Activity in Saliva of Children with Ariboflavinosis.

Group	No. of case	Age (years)	Lingual change	Angular stomatitis	Cheilosis	Marks for B_2-deficiency	Lysozyme activity in saliva (%)	
V	78	8	−	++	++	4	68	50.8±8.1
	79	8	+	++	+	4	57	
	80	8	++	+	+	4	55	
	81	8	−	++	++	4	55	
	82	10	++	−	++	4	54	
	83	10	+	++	+	4	35	
	84	10	+	++	+	4	27	
	85	11	+	++	+	4	29	
	86	11	+	+	++	4	72	
	87	11	+	++	+	4	28	
	88	12	+	+	++	4	69	
	89	12	+	+	++	4	57	
	90	12	+	+	++	4	75	
VI	91	8	++	++	+	5	57	56.2±12.0
	92	10	+	+	+++	5	52	
	93	10	++	++	+	5	41	
	94	11	+	+	+++	5	60	
	95	12	+	++	++	5	71	
VII	96	10	+	++	+++	6	62	62.5±7.0
	97	10	++	+	+++	6	65	
	98	11	+	+++	++	6	59	

As regards salivary lysozyme, an increased activity was reported in cases of gastric ulcer,[4] tuberculosis,[5] and allergic diseases,[6] whereas a decreased activity was found in otitis media, trachoma, and neurasthenia.[7] Among the

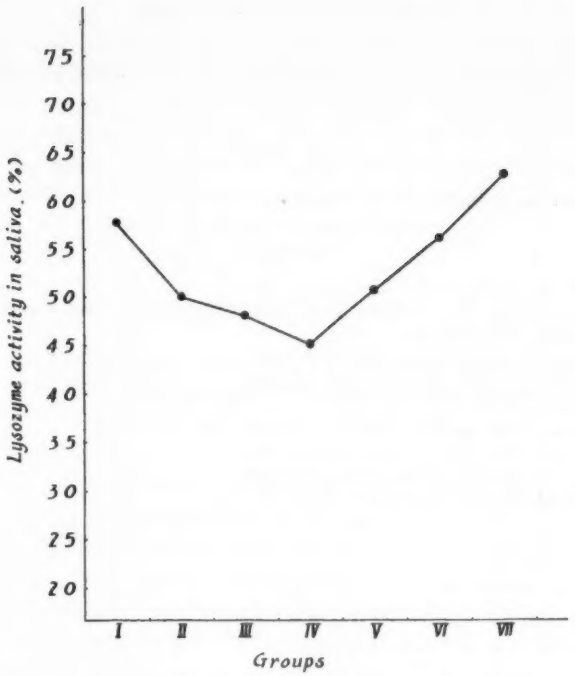

Fig. 2. Averages of salivary lysozyme activity in groups I~VII.

rest, Burghartz *et al.*[6] reported that lysozyme activity in saliva was decreased by an administration of ACTH or cortisone. This was very impressive for me, because a possibility of adrenocortical hyperactivity resulting from ariboflavinosis had been suggested by Ishioka[8] in our Laboratory, who considered the low sodium-potassium ratio in saliva of the majority of ariboflavinotic boys as an evidence of adrenocortical hyperactivity — at least from the view point of salt-regulating hormones.

According to Skelton[9] riboflavin deficiency in itself acted as a non-specific stress on rats. As is well known, an exhaustion of adrenocortical activity occurs in a later stage of long continued stress, and this will hold good in B_2-deficiency in human being, because atrophy of adrenal cortex has been one of common findings in autopsy of pellagrins.[10]

Taking into consideration the criteria above mentioned, it is probable that a decreased activity of the salivary lysozyme in Group VII or the cases of extremely severe ariboflavinosis was related, to some extent, to adrenocortical exhaustion.

SUMMARY AND CONCLUSIONS

Lysozyme activity in saliva, estimated by using its lytic action upon micrococcus lysodeikticus, showed a tendency to increase in ariboflavinosis of slight to moderate degree, and to decrease in extremely severe ariboflavinosis. The fluctuation of the salivary lysozyme activity was probably influenced by functional state of the adrenal cortex to which the grade of severity of ariboflavinosis is probably closely related.

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Salivary Lysozyme: Its Relation to Angular Stomatitis in Ariboflavinosis

Part II.

Lytic Action of Lysozyme upon Micrococcus Lysodeikticus Cultured with or without Riboflavin

By

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(Received for publication, June 28, 1961)

As has already been shown by Fleming,[1] micrococcus lysodeikticus succumbs easily to lytic action of lysozyme.

A low activity of salivary lysozyme in boys of slight to moderate ariboflavinosis was identified in my previous investigation.[2] In the present paper I desire to show an influence of riboflavin added to culture media of micrococcus lysodeikticus upon susceptibility of the microorganism to the lytic action of lysozyme.

MATERIALS AND METHODS

Composition of culture media for micrococcus lysodeikticus

Four kinds of media were used (Media I–IV). With exception of riboflavin, the composition of Media II–IV was the same as that of Medium I.

Medium I : Agar was added in concentration of 2.5% to Kuwahara's liquid medium[3] for staphylococcus. No riboflavin was contained in it (cf. Table I).

Medium II : Riboflavin was added in a concentration of 1 mg per 100 cc of Medium I.

Medium III : Riboflavin was added in a concentration of 2 mg per 100 cc of Medium I.

Medium IV : Riboflavin was added in a concentration of 3 mg per 100 cc of Medium I.

Successive transplantation of micrococcus lysodeikticus upon each of Media I–IV

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Table I. Composition of Medium I (or Medium Kuwahara).

Tryptophane	0.01g	NaCl	5.0g
Cystine	0.01g	$MgSO_4 \cdot 7H_2O$	0.2g
Casein hydrolysate	1.0g	KH_2PO_4	0.35g
Glucose	1.0g	$Na_2HPO_4 \cdot 12H_2O$	2.5g
Nicotinamide	1.23mg	Agar	25.0g
Thiamine	0.033mg	Distilled water up to 1000 cc (pH 7.2)	

Micrococcus lysodeikticus cultured with the ordinary agar plate was transplanted on a slant agar of Medium I, and cultured on it for 48 hours at 37°C —the first generation of the micrococcus on Medium I. The microorganism thus cultured was transplanted upon another slant agar of Medium I and cultured for 48 hours at 37°C — the second generation on Medium I. In such a way the transplantation and culture were carried out until the 20th generation on Medium I was obtained.

Such transplantation and culture as were carried out in the case of Medium I were simultaneously performed in the cases of Media II, III and IV. The generations of from the first to 20th on each of Media I–IV were thus obtained.

Estimation of susceptibility of micrococcus lysodeikticus to lytic action of lysozyme

Principle of estimation: If concentration of lysozyme and that of micrococcus lysodeikticus to be tested are kept being fixed, dissolution of the microorganisms will take place in large or small extent corresponding to high or low susceptibility of the microorganisms to lytic action of the lysozyme respectively.

For the purpose of a numerical expression of susceptibility of the microorganism to lysozyme, I adovocated the term "percentage of dissolved microorganisms", that meant a percentage of an amount of the dissolved microorganisms as compared with that of the microorganisms which existed prior to being subjected to lytic action of the lysozyme.

Details of procedure

1) "Micrococcus suspension of original concentration": A micrococcus suspension in M/15 phosphate buffer of pH 6.8 was so prepared as to get a concentration of 50 degrees of optical density at 640 mμ by Leitz-photometer.

2) 4 mg% solution of lysozyme (Worthington Biochem. Co. U.S.A.)

3) Technique

5.0 cc of "the micrococcus suspension of original concentration" were put into each of two test tubes (tube T and tube C), then 0.5 cc of the lysozyme solution was added to tube T, while 0.5 cc of the phosphate buffer was added to

tube C. The content of each was mixed well and kept at 37°C for 12 hours. After incubation, optical density of the content of each of the tubes was read at 640 mμ by Leitz-photometer. Suppose optical densities: O_T and O_C were found in the cases of tubes T and C respectively, then "the percentage of the dissolved micrococcus" could be calculated according to the following formula: $(1-O_T/O_C) \times 100\,\%$.

RESULTS AND DISCUSSION

In the above experiments, "the percentage of the dissolved micrococcus" was the same in the first generation in all the cases of Media I, II, III and IV,

Table II. Percentage of dissolved microorganism at Various Generations on Media I-IV.

Generation / Media	Percentage of dissolved microorganism				
	1st	5th	10th	15th	20th
Medium I	39	38	39	39	40
Medium II	36	31	26	25	25
Medium III	38	29	23	21	21
Medium IV	36	30	21	19	19

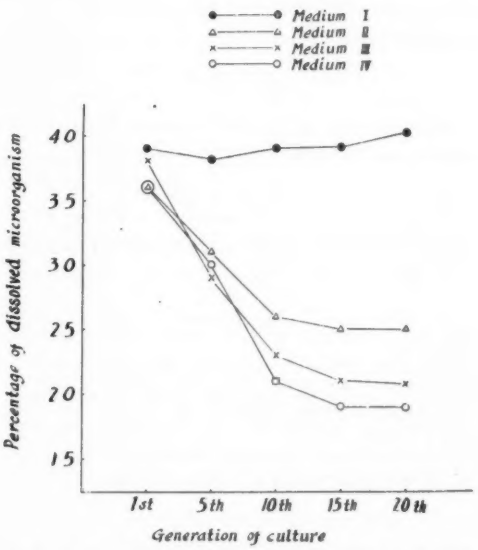

Fig. 1. Percentage of dissolved microorganism at various generations on Media I~IV.

while in and from the 5th generation downwards it began to decrease remarkably in the cases of Media II, III and IV, then a tendency of the decrease was somewhat different according to the different media, the most conspicuous being Medium IV, then Medium III, the least conspicuous being Medium II. From these results it was highly probable that micrococcus lysodeikticus became more resistant against lytic action of lysozyme when it was maintained on riboflavin containing media as compared with the case in which it was cultured with riboflavin-free medium, and that the larger the riboflavin content in the media, the more resistant became the microorganism against lytic action of lysozyme.

Since the first report of Fleming[1] concerning a lytic action of lysozyme upon micrococcus lysodeikticus, several authors investigated the mechanism through which the microorganism was dissolved by lysozyme. According to Epistain *et al.*[4] and Boasson[5] a certain alteration of polysaccharides of cell membrane of micrococcus lysodeikticus was brought about by lysozyme with the final dissolution of the microorganism.

Matsunaga's report[6] was, as far as I know, only one dealing with the susceptibility of micrococcus lysodeikticus from view point of composition of culture media of the microorganism. Matsunaga[6] used the Kuwahara's medium[3] and found that the susceptibility of the microorganism was increased when the medium was deprived of thiamine or nicotinamide. But in his report no discription was found concerning riboflavin as an ingredient of culture media for micrococcus lysodeikticus.

REMARKS

Susceptibility of epithelial cells from lips of boys with ariboflavinosis to lytic action of lysozyme will be reported in Part III.

CONCLUSIONS

Riboflavin added to culture media of micrococcus lysodeikticus reduced the susceptibility of the microorganism to lytic action of lysozyme.

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Use of Buccal Varidase in the Postanesthetic Period

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Varidase, an enzymatic preparation containing streptokinase and streptodornase, has heretofore been used mainly topically for the purpose of enzymatic débridement in suppurative, inflammatory conditions, but recent investigation has revealed that it can be applied systematically in a form of buccal tablet[1] and the indication has been extended as a beneficial adjunct to various surgical procedures,[2] traumas,[3] as well as otolaryngeal[4] or dental surgery[5] because of its activity in reducing inflammatory edema. Being interested in the fibrinolytic activity and the ease of administration of Buccal Varidase, we use it in surgical patients in the postanesthetic period to facilitate expectoration of viscid bronchial secretions. Administration of general anesthesia may be followed by an increase in respiratory tract secretion. When suptum is viscid it is often difficult for postoperative patients to expectorate because coughing produces pains at the site of operative wounds. Failure to expectorate and retention of sputum are likely to cause postoperative pulmonary complications, particularly atelectasis.[6] Consequently, it is of great importance that expectoration be facilitated as soon as possible after surgery in order to avoid sputum retention and pulmonary complications. In this paper the results are presented of the effect of Buccal Varidase in the postanesthetic period from this point of view.

METHODS

In this study we selected only those patients who either complained of difficulty in expectorating sputum or had a history of such difficulty after previous surgery. The subjects were 100 in total (male 57 and female 43) with the age range from 6 to 80 years. After an ordinary premedication with meperidine and atropine anesthesia was maintained with nitrous oxide, oxygen and ether or fluothane under endotracheal intubation. Each patient was given one tablet of Buccal Varidase (10,000 units streptokinase, 2,500 units streptodornase) every 6 hours for one or two days in the postoperative period.

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About two-thirds of the patients underwent abdominal operation. Total tablets administered are shown in Table I.

TABLE I.

Tablets administered in total	Number of cases
1	1
2	2
4	72
6	5
8	17
12	2
20	1

RESULTS

In the majority of cases the effect of the medication began to become apparent within 60 minutes after the administration of one or two tablets. Thick, viscid sputum became thin and serous, which could be expectorated very easily. In 87 cases the result was good and satisfactory. They became completely free from the distress due to difficult expectoration after the administration of 4 to 8 tablets. In the remaining cases the result was fairly good and the distress of the patients was considerably alleviated. In some cases it was necessary to perform tracheostomy and frequent suction, when bronchial secretions were too much. Improvement of dyspnea was very impressive in some cases. Undesirable side-reactions such as stomatitis, heartburn, nausea or allergic reactions were not encountered in this group of patients. Abnormal hemorrhagic tendency or disturbance of wound healing was not observed in clinical cases as well as in animal experiment. Some illustrative cases are shown in the following.

Case 1:

A 61 year-old woman underwent arthroplasty of the left elbow joint under general anesthesia. On the next day the patient became dyspneic and restless with chest pains, apparently due to the accumulation of sputum. One tablet of Buccal Varidase was given, then within 60 minutes sputum became remarkably thin and expectoration became loose and easy. Following the administration of the tablet every 6 hours improvement of dyspnea was very impressive and she recovered completely from the complaint after two days.

Case 2:

A patient, 50 years old man, underwent colectomy for carcinoma, when he was greatly distressed by difficult expectoration of viscid sputum. About one month later he was operated on again for postoperative intestinal adhesion. This time the buccal tablet was given every 6 hours after the operation. Sputum was expectorated very easily and he was completely free from the previous complaint in the second postoperative course.

Case 3 :

A 69 year-old man was operated on for rectum carcinoma. Two weeks after the operation the patient became dyspneic, probably due to pulmonary complications. He was in a serious condition. Tracheostomy was done and he was moved in an oxygen tent. Bronchial secretions were too viscid to suck out from the tracheostomy. Following the administration of 4 tablets of Buccal Varidase, the secretions became serous and were easily eliminated by suction. Dyspnea improved considerably after the administration of 8 tablets.

Case 4 :

A 43 year-old woman developed dyspnea, accompanied with chest pains and cyanosis, on the second day after supravaginal amputation of the uterus. On the third day she was admitted to our hospital in a serious condition. Massive atelectasis of the right lung was suspected from X-ray examination. Immediately she was laid in an oxygen tent and tracheostomy was performed, but suction was almost ineffective because secretions were too viscid. Buccal Varidase was given every 6 hours with antibiotics. After 4 tablets sputum became thin and serous and was eliminated easily through the tracheostomy. The condition of the patient improved remarkably after the administration of 8 tablets. The X-ray photographs of the chest following the course were shown in Fig. 1. She recovered uneventfully and was discharged on the 18th day.

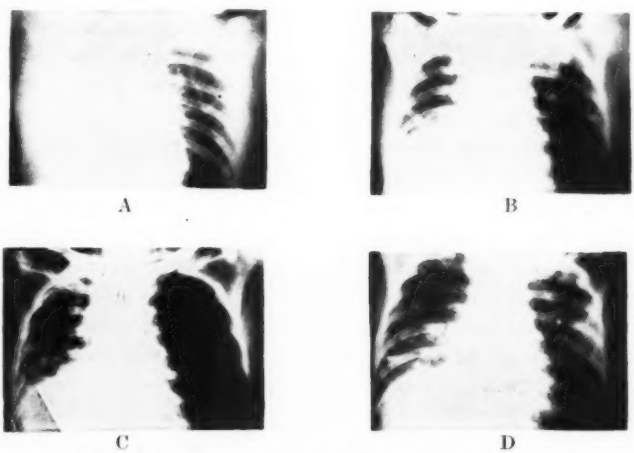

Fig. 1. X-ray photographs of the chest in Case 4 following administration of Buccal Varidase (one tablet every 6 hours, 8 tablets in total)

A : on the day of admission, before Varidase treatment
B : 24 hours after start of Varidase treatment
C : 48 hours after start of Varidase treatment
D : 72 hours after start of Varidase treatment

Varidase may influence blood clotting time because of its fibrinolytic activity, but no significant increase in blood clotting time was observed when it was examined in 15 patients at 24 and 48 hours after the start of Varidae treatment. The influence of Varidase upon wound healing was also investigated in rabbits. Macroscopic observation revealed no significant differences in wound healing between control and treated animals, but microscopic examination showed a slight tendency to inhibit cellular reaction at the site of the wound, when Varidase was given 20 times as much as an ordinary dose. In clinical use, however, Varidase does not seem to causes any deleterious effect upon wound healing.

DISCUSSION

Although the indications of Buccal Varidase have been extended widely in surgical as well as in medical fields, no informations were available so far of its application to patients in the postanesthetic period with special purpose to facilitate expectoration of viscid sputum. In the postoperative period it is not uncommon that thick, viscid sputum causes a considerable distress to the patients. Failure of expectoration and retention of sputum are known to be responsible as a main causative factor for postoperative pulmonary complications, particularly atelectasis. The present study was undertaken to evaluate Varidase in the postanesthetic period. The results obtained were very satisfactory. Soon after the administration of one or two tablets sputum became serous and coughing became loose and productive. In the majority of cases the administration of one tablet every 6 hours for one or two days seemed enough to alleviate the complaints of the patients. But in some cases it was necessary to do tracheostomy and frequent suction, when sputum was too much. No significant local or systemic side-reactions were encountered. Blood clotting time examined so far in this series showed no abnormalities. But because of its fibrinolytic activity it seems wise to avoid its application in those patients who have hemorrhagic tendency, liver diseases or hypoproteinemia.

SUMMARY

Buccal Varidase was administered to one hundred postoperative patients who either complained of difficult expectoration of viscid sputum or had such difficulty in previous surgery. The agent proved to be effective in facilitating expectoration in the majority of cases. No undesirable side-reactions were encountered in this group of patients. Buccal Varidase seemed to be a drug of choice in alleviating the complaints of the patients due to difficult expectoration and in reducing pulmonary complications in the postanesthetic period.

Buccal Varidase was supplied by the Lederle Laboratories, a Division of American Cyanamid Company, Pearl River, New York, U.S.A.

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